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Outlook with Labour

MR WILSON has eventually secured what most expected him to, and the political outlook, precarious as it is, is unlikely to force him into another election before he has had time to govern for six months to a year.

It was apparent immediately Mr Wilson announced his Cabinet that this was not to be a palliative government, shaped to defuse the Liberals and other parties. Three key posts, Employment, Industry and Trade go to three left-wingers Messrs Foot, Benn and Shore—vigorous proponents of nationalisation and renegotiation of the European Treaty. Unlikely as it is that moves towards nationalisation will come in the next few months, it is almost certain that within a year this Labour Government would be moving towards public ownership in several industries.

The three appointments that will most interest British scientists are those of Mr Roy Mason to Defence, Mr Eric Varley to Energy and Mr Reg Prentice to Education and Science.

Defence isn't what it used to be. The great debates on Britain's nuclear deterrent and her world posture are now part of history and there is a deep lack of interest in defence problems. The only highly visible role of the armed forces is in Northern Ireland, the last and most insoluble of the colonial peace-keeping problems. This is an operation that many tough-minded citizens would like to see terminated 'to let the Irish sort it out for themselves', and certainly few outside military circles give military matters much thought.

This is a bad situation. The military mind left to its own devices will expand outrageously. Mr Mason is committed by the Labour Party's manifesto to prune hundreds of millions of pounds off defence spending and will meet fierce resistance. In the past two years military research and development expenditure, having declined steadily for years as a fraction of total military expenditure, suddenly started to rise again. Much is in the pipeline, such as the Multi-Role Combat Aircraft and improvements to the Polaris fleet. Mr Mason will, no doubt, squash plans for the refitting of Polaris. He might well start enquiries into the long term future of anti-submarine warfare and nuclear weapons development.

Mr Varley comes into a young ministry and has many well-wishers. Not, as one newspaper put it, because "his mining experience should be useful" as if years of handling coal gave one a special insight into energy problems shared only by petrol-pump attendants. Rather, his is a relatively unknown face and there is hope that a fresh mind, not too blunted by previous political battles, could do a good job in this demanding ministry.

The first major issue that he will have to consider is the nuclear reactor question. This is one of those de-

cisions in politics which despite all the finely calculated analyses and extensive documentation will be made on instinct. Noises before the election suggested that the instincts are rather strongly against light water reactors, and in favour of the very cautious step of more magnox reactors.

Mr Prentice is rather a surprise at Education and Science. He had been at the ministry before at a junior level, but has recently been 'shadowing' employment. However, his well-known dislike of the left-wing made that an impossible post for him. Education and Science may still only be a temporary staging post.

It is most unlikely that science will see any major changes in the year ahead other than those caused by financial constraints. Labour has no plans for altering the present relationship between research councils and the research they sponsor, so for the time being the Rothschild restructuring will presumably be allowed to proceed. Labour likewise does not intend to implement the recent recommendations for student loans and for changing the emphasis to post-experience study for PhDs.

Finally, what of some of today's symbols? Concorde, child of the white hot technological revolution of the 1960s, will no doubt fly for BOAC and Air France and few else: this is a business decision and politicians will be glad to be rid of it. Maplin, London's third airport, will have to wait. And the think tank? One suspects it will stay, and that one or two of *Nature's* readers will end up in it.

100 years ago



IN a most interesting article on the planet Mars, in your issue of *NATURE* for Feb. 19, which has just been shown to me, the Rev. T. W. Webb directs attention to the question of the colours of Mars being due to effects of contrast or not, and says—"Nor does it seem to have been noticed that no effect of contrast has been traced in the Polar snows."

Kindly permit me to inform Mr. Webb that, in a paper on Mars in the last volume of the "Monthly Notices of the Royal Astronomical Society," I expressly state that, "on May 14, 1873, the south Polar ice appeared (in an 8½-inch silvered glass reflector, by Browning) of quite a pale sky-blue colour, evidently by contrast," and I may add that this effect I noticed also on two or three subsequent occasions.

Burton-on-Trent, March 12

EDWARD B. KNOBEL

From *Nature*, 9, 247, February 12, 1874.



Scientists' dispute is symptom of a deeper problem

Dr G. J. Leigh, of the Agricultural Research Council Unit of Nitrogen Fixation at the University of Sussex, expresses his personal views about the pay dispute involving British Civil Service scientists.

THE current agitation among Civil Service scientists is stimulated by comparisons of pay scales such as those in Table 1. Scientists have been in dispute with the Civil Service Department (CSD) for some four years but I shall not detail the history of the dispute here. Its resolution currently lies with the Pay Board but is not yet in sight. It is the CSD's opinion that the question is not one of relationships and Lord Windlesham has now implied that the government is not bound to accept the findings of the Pay Board. In November 1973, at the inception of Stage III, the CSD negotiated with indecent haste a pay settlement for about 400,000 civil servants, but excluding scientists, giving increases of nearly 20%, and there is a feeling among scientists that the CSD is conducting a vendetta against them. How else to explain the situation in which an officer with 3 A-levels can qualify for the Family Income Supplement?

The current situation is, however, a reflection of conditions which have existed for many years. The government employs more scientists than any other organisation in the country yet it is far from clear whether the government—any government—has ever had a coherent policy for science and technology other than allowing developments to occur by default. Any government of a modern state requires scientists to carry out routine tasks but it has been accepted for more than fifty years, since Haldane at least, that the British government should sponsor general long term research and have available a source of informed scientific opinion. Presumably this requires a pool of scientists of the highest quality. But although an individual can define areas in which he feels the government should be active, there is little indication that a government knows how it should use its scientists or even that it really wants to.

The 1964 Labour government talked about the white heat of technology but the only long lasting product of the

conflagration would seem to be Concorde. The 1970 Conservative administration carried through the Rothschild exercise. And it cannot be denied that any group which consumes large amounts of state money has to recognise that it is accountable to the state. Scientists are perhaps more cautious and conservative than most but the manner of introduction of Rothschild principles was not calculated to allay their apprehensions. One consequence was the creation of uncertainty among a large group of scientists who felt they were being forced into a new kind of organisation of which they did not necessarily approve. Another was to enlarge the bureaucratic machinery through which research projects must filter before being acted on.

Lord Rothschild did not attempt, however, to define the areas in which applied research should be carried out. He left the selection of work area to a rather artificial market mechanism such as has operated (sometimes with conspicuous lack of success) for defence contracts. Nor did he deal with pure research, which was outside his immediate terms of reference. Consequently, research programmes must become dependent on specific pressures from industry or ministries, and for that reason are likely to become primarily short term in nature.

Nevertheless, the Rothschild mechanism does represent a way in which a government could introduce a broad coherent policy covering both pure and applied research, and encompassing problems such as cancer, fuels or resources. It also implies a scientific Civil Service which has a properly developed structure in which problems such as pay relationships, short term contracts, transfer between Civil Service classes and career development in small research units, have been fairly resolved.

The reality is rather less encouraging. For example, one hears through the grapevine that there is money 'available' for research into energy and resources problems. It would seem that this will produce some postgraduate and post-doctoral research in a few university departments rather than be applied in a broad, considered approach to national problems. The response is too little, too timorous and probably too late.

The application of the Fulton principle of unified pay and grading in the Civil Service, which would solve many of the problems outlined, has been supported by both Labour and Conservative governments. Even while it has been official government policy, the consequence of the CSD's actions has been to make the implementation below the senior grades more difficult. Ministers have expressed deep regrets about the current situation but nothing more concrete has transpired. Consequently Civil Service scientists have become much more aware of the need to organise themselves and of the need to have a strong trade union,

TABLE 1 Relative pay scales for Civil Service groups at January 1, 1974 and numbers in post on April 1, 1973

Science Group	No.	Salary range (£)	Administration Group	No.	Salary range (£)
Principal Scientific Officer	2,140	4,895-3,715	Principal	3,778	5,775-4,360
Senior Scientific Officer	3,505	3,895-2,798	Senior Executive Officer	6,403	4,542-3,756
Higher Scientific Officer	3,904	2,854-2,221	Higher Executive Officer	19,996	3,585-2,953
Scientific Officer	2,898	2,329-1,410	Executive Officer	45,670	2,782-1,819
Assistant Scientific Officer	3,800	1,729- 792			2,306-1,360
Subtotal	16,247		Total	75,847	
In fringe bodies (for example					
United Kingdom Atomic Energy					
Authority research councils)	11,206				
Linked grades (for example					
Patent examiners, Defence College					
lecturers)	4,501				
Total	31,954				

Experimental Officer and Scientific Officer and Assistant Scientific Officer are not exactly comparable

whether inside or outside the Trades Union Congress.

I have tried to show that the current pay dispute is only a symptom of a much deeper problem, which is how (and possibly whether) the government should use science and scientists. Certainly scientists will have to learn to be more adaptable but they cannot be treated as operatives

who perform a service to order. It is the duty of a government, through its science policy, to create the environment in which scientists can work constructively. It is in the interests of scientists and the community in general that the current impasse be resolved and not be allowed to re-develop.

Picture by Robin Laurance

Scientists on the march

Four thousand government scientists and a labrador dog, led by a jazz band playing the blues, made up the first major demonstration to converge on Number Ten, Downing Street, during Mr Wilson's latest tenancy. After a mass meeting at the Central Hall, Westminster, where striking scientists signalled their militancy with well ordered applause at their general secretary's every cadence, the demonstrators bore down on the Prime Minister, and at one stage their parade filled Whitehall from one end to the other. The occasion was the first national half day strike by members of the Institution of Professional Civil Servants (IPCS), whose campaign to force a decision on their long delayed pay claim happened to coincide with a general election campaign. Indecision over the state of the parties, and their relative abilities to form a government, left the scientists with a grand scale protest planned and no minister in office to enjoy the benefit of it. So they settled for Mr Wilson, who on a bright afternoon, to the strains of the St Louis Blues, received the following note:

"You will know that civil service scientists and those employed in fringe bodies have today been on strike in connection with the completely unreasonable delay in dealing with their long outstanding pay claims. This is the first occasion on which any group of the institution's members have taken national action of this kind, and it is the clearest possible demonstration of their dissatisfaction with the present position. At a meeting of scientists employed in the London area, at which something like 2,000 were present, the following resolution was carried: this mass meeting, representing thousands of scientists employed in the Civil Service and fringe bodies in the London area, expresses its deep sense of outrage that after three years of patient endeavour the government is still procrastinating on scientists' reasonable pay demands. It stresses the absolute necessity for immediate pay increases for scientists who are unique in having suffered a fall in living standards over the past three years and who are now at least 28% behind general pay movements. It urges the government to give priority to producing a fair and satisfactory solution to this urgent problem."

The Pay Board's report on scientists' pay, the delay of which caused the IPCS to mount a campaign of industrial action, is expected under the new administration to be published rather promptly. Not only does Mr Wilson have a distinct commitment to placate angry unions, but the Pay Board, whose future may well hang in the balance, does not have much else to work on these days. The SOS placard in the pictures is a new slant on a slogan which the outgoing government coined in order to save power during the miners' strike: "Switch off something". The electorate apparently got the message.



international news

Academy reports on Vietnam herbicide damage

Colin Norman, Washington

IN the late 1960s, United States military forces dumped nearly 19 million gallons of herbicides on an area of South Vietnam equal in size to the entire state of Connecticut or Northern Ireland. According to the National Academy of Sciences, the most prestigious scientific organisation in the United States, some of the sprayed areas could bear the scars of this massive chemical assault for 100 years or more unless swift action is taken to reforest the land. The academy has also reported that interviews with people living in the highlands of South Vietnam have produced extremely consistent reports that children and animals were killed by the herbicide sprays.

Those conclusions have emerged from an intensive study conducted by an international team of scientists which worked for three years under the academy's auspices. After much delay and considerable wrangling in the academy itself, the fruits of the team's endeavours were published last week in the form of a 400-page report* prepared for the Department of Defense and the US Congress. The document provides the most extensive and authoritative survey so far of the damage caused to the Vietnamese people and their environment by military herbicide operations, but it is unlikely to quell the bitter debate that has been going on in the United States about this aspect of the Vietnam war.

For one thing, the report is already being used by both sides to bolster their cases. The Department of Defense, for example, has put out a statement which invites the reader to draw the following conclusion from the report: "some damage has resulted from the military use of herbicides in Vietnam, however, most of the allegations of massive, permanent ecological damage are unfounded". On the other hand, it has been argued that

the academy has turned up evidence of damage so extensive that it provides a compelling case for a flat ban on the use of herbicides in war.

Another reason why the report is unlikely to dampen the debate is that the investigating team has failed, for a variety of reasons, to come up with a definitive answer to the most contentious question of all—whether or not the herbicide spraying caused an increase in the number of birth defects in communities that were particularly hard hit.

Finally, the team itself failed to reach agreement on how much destruction has been wrought on Vietnam's inland forests. This aspect, in fact, held up publication of the report, caused three members of the team to disassociate themselves from the final conclusion, and is sure to lead to a long dispute.

The report, nevertheless, is an extremely valuable document, not least because it is the first authoritative account of the extent and nature of the military herbicide missions flown over South Vietnam between 1965 and 1970. In all, nearly 9% of the area of South Vietnam received at least one dose of herbicide.

Working from a military record which gave details of the time, place, type and amount of herbicide sprayed, in conjunction with vegetation maps and aerial photographs, the academy team constructed a picture of the spray operations which indicated that 10.3% of South Vietnam's inland forests, 3.2% of the cultivated land, 36.1% of the mangrove forests and 5.5% of other areas in the country were doused with a variety of herbicides at least once.

In the total of nearly 19 million gallons of herbicide distributed over South Vietnam, the majority—11.22 million gallons—was so-called 'agent orange'. This is a half-and-half mixture of the herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). Use of this agent was terminated in 1970, according to the military records, because it had been suggested that 2,4,5-T may cause birth defects, and because it was found that the stuff was contaminated with a highly toxic chemical called TCDD (2,3,7,8-tetrachlorodibenzo-para-dioxin). The other two chief agents used were 'agent white' (2,4-D and Picloram) and 'agent blue' (sodium cacodylate, and arsenic compound).

According to the military records, about 88% of the spraying was directed at forests, 9% was aimed at destroying crops in areas under the control of the National Liberation Front (NLF), and about 3% as directed at the perimeters of military bases. But those figures underestimate the area of crops destroyed for a variety of reasons. For one, spray from defoliation missions tended to drift outside the target area on to crops; in fact, the report states that "in 16 out of 18 areas studied by aerial photography, crop damage by defoliation missions was greater, and sometimes considerably so, than by crop destruction missions". For another, the Montagnards (the highlanders) conduct much of their agriculture in cleared areas of forests, and so their fields were often sprayed during attacks on the inland forests. Finally, the report suggests that in some defoliation missions, herbicide was in fact sprayed directly on crops, although the investigating team is careful to say that their suggestion "is not intended as an expression of opinion as to whether or not the official policy on herbicide operations was violated".

The damage wrought by this assault varied considerably from one area to another, but the most severe and the most persistent destruction was suffered by the mangrove forests. The report indicates that a single spray attack on a mangrove forest was sufficient to kill all the trees directly hit, and some regions of South Vietnam have been completely denuded by repeated herbicide applications.

More than a third of all the mangrove forests in South Vietnam were directly sprayed, according to the records, and the report states that "large contiguous areas were devastated, and there has been little or no recolonisation of mangrove trees in extensive areas". The reason is that some regions, such as the Rung Sat zone southeast of Saigon, were so heavily sprayed that all the vegetation has been destroyed and no seed sources are left to colonise the area. The report states that "under present conditions of use and natural regrowth, it may take well over 100 years for the mangrove area to be reforested". A massive reforestation programme, however, could probably restore the mangrove forests in two or three decades.

Although those reports are bad enough, some observers have suggested

* *The Effects of Herbicides in South Vietnam, Part A*: Available from the National Academy of Sciences, 2101 Constitution Ave NW, Washington, DC 20418. \$10.

that they may even underestimate the extent of damage to the mangrove regions. Dr Matthew Meselson, a Harvard biologist who surveyed the effects of herbicide damage for the American Association for the Advancement of Science, suggested in a telephone interview last week, for example, that uncertainties about the extent of the area sprayed, drifting outside the target area, and other factors may have caused the academy team to underestimate the sprayed area by about 30%. In any case, Dr Philip Handler, President of the academy, points out in a personal introduction to the report that unless a vigorous reforestation programme is conducted in the mangrove forests "mankind will have been guilty of a large and ugly depredation of our natural heritage".

Although the academy team and other observers are in relatively close agreement about the massive devastation suffered by the mangrove forests, the effect of herbicide spraying on inland forests is open to considerable debate. Because much of the survey was carried out while the war was going on, and because even after the cease-fire was signed a considerable amount of fighting was still taking place, the academy team had to rely chiefly on aerial photography for its analysis of the destruction of inland forests. Under those conditions, little ground work was possible. Consequently, the data are not of the best quality, and their interpretation is, to put it mildly, a difficult task.

When the report was in one of its early drafts, it became apparent that the investigating team's estimate of the destruction was considerably below that of other observers and investigators who had surveyed the damage for themselves. The academy's Report Review Panel, a committee of academy members which screens publications before they are released, refused to accept the estimates, and instructed the team to take another look at the data. It later became apparent that the extent of damage was underestimated because little account had been taken of the fact that repeated herbicide applications killed the trees to a greater extent than the team had first supposed.

Three independent photointerpreters and forestry experts were called in to help with the analysis, and the report eventually came up with the suggestion that about 1.25 million cubic metres of 'merchantable' timber had been lost from the inland forests because of herbicide spraying. The report said that although the exact figure cannot be taken as Gospel truth, it almost certainly lies between 0.5 and 2.0 million cubic metres.

Two of the independent photointerpreters reckoned that the figure is probably near 2.0 million cubic metres, while the third said that it could be an order of magnitude greater. One of the team, Professor Pham-Hoang-Bo from Saigon University, dissented from the report because he believes that the estimate of damage to the inland forests is much

too low. Another member, Professor Paul Richards of the University College of North Wales, dissented for similar reasons. Professor Alexander Leighton of the Harvard School of Public Health, said that he couldn't go along with the conclusion because he has no expertise in the field. And Dr Meselson, who was a member of the report review panel, said last week that he believes that the report's estimate of tree damage understates the case by at least an order of magnitude.

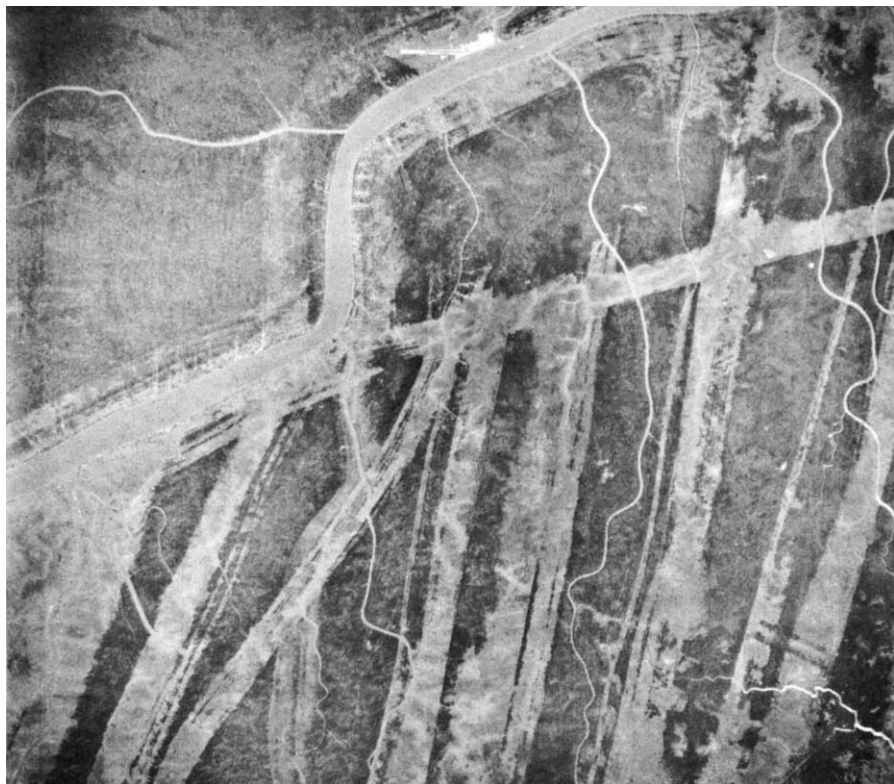
Meselson pointed out for example that the report gives a figure for the density of trees in Vietnam forests which is considerably less than the density of trees on the front lawn of the National Academy of Sciences—a lush, very lightly wooded expanse of grass. He said that this shows that there is clearly something wrong with the interpretation of the photographs, and he would like to see the academy team brought back together "if they can bear it" to take yet another look at the data. In any case, he said that he intends to publish his own conclusions independently.

Whatever the final outcome of the debate about the extent of the devastation of the inland forests, the academy report suggests that in lightly sprayed areas, redevelopment will probably resemble the pattern of forest growth following harvesting of large trees. The more heavily sprayed areas are, however, another matter, and will require considerable help in reforestation. It is reckoned that about 12% of the total sprayed area will require a major assistance programme, but Meselson believes that the figure is considerably greater.

As for the effects of herbicides on the people of Vietnam, the academy team looked for toxicity to people directly exposed to the herbicides, birth defects in the offspring born to exposed mothers, economic effects from crop destruction, and the psychological effect of the whole military herbicide programme on the Vietnamese people.

The most disturbing accounts of damage from acute toxic effects came from interviews with Montagnard people who live in the highland regions. The interviews, mostly conducted by Dr Gerald C. Hickey of the Southeast Asia Program of Cornell University, produced remarkably consistent reports of death to children, diarrhoea, skin rashes looking like insect bites, and abdominal pain following spray missions. They also reported widespread deaths among their animals, particularly chicken and pigs, and they said that the spraying killed fish in the streams.

Dr Hickey said in a telephone interview last week that he first heard about the reports of deaths from Montagnards in refugee camps, but he later went into



Aerial photograph of damage to mangrove forest in Ca Mau Peninsula in southern tip of Vietnam. Photo taken in 1971 shows effects of individual spraying missions over the forest. (Reproduced by permission of the National Academy of Sciences.)

the villages to talk to the people in their own localities. The reports, he said very accurately described the spray planes, the effects of the herbicides on the vegetation, and the accountants of the toxic effects on humans were consistent from village to village. The report itself says that "reports of illness following spraying are so striking it is difficult to dismiss them as simply the effects of propaganda, high normal death rates, or faulty understanding of cause and effect".

Asked why the reports of death came mostly from the highland people, Hickey suggested a number of possible reasons. First, they were hit mostly with agent orange, perhaps the most toxic of the herbicides used in the military operations, particularly since it is contaminated with TCDD. Second, the defoliation missions wiped out their crops because their fields are mostly in areas of reclaimed forest, and this tended to increase exposure to the chemicals. Third, the Montagnards tend to live out of doors much more than the lowlanders do, and they carry their children on their backs when they work in the fields. Thus they would be more likely to be exposed to the herbicide sprays. And finally, the people repeatedly emphasised in their interviews that they "didn't know what was happening" when the aircraft started spraying, and many of the children drank contaminated water or ate sprayed crops.

Since the reports came from direct interviews with Montagnards, they are difficult to verify by independent means, but Dr Hickey, a world renowned authority on the Montagnard people, is extremely disturbed by them. He is also extremely disturbed by Dr Handler's personal introduction to the academy report, which labelled the accounts as "secondhand" and "tales". Handler also said that the committee regrets that it was unable to visit the Montagnards in their own regions to verify the reports. Hickey did, however, get his information firsthand (albeit with the help of interpreters), and he did follow up his investigations in the Montagnard villages. He said last week that he had no chance to see Handler's introduction, in fact he didn't even know it would be written, and said that he finds it "outrageous". He said that in his nine years in Vietnam, he has encountered innumerable attempts at covering up the damage wrought by the war, and that this "is another one of them".

As for the effects of herbicides on birth defects and stillbirths, the report explicitly states that the team "could find no conclusive evidence of association between exposure to herbicides and birth defects in humans". The basis for that comment was a search through the records of two major Saigon hospitals

which showed "no consistent pattern of association between rates of congenital malformations and annual amounts of herbicides sprayed".

But a more extensive study is being conducted on the records of the Childrens Medical Relief International unit (the so-called Barsky Unit) of the Cho Ray Hospital in Saigon-Cholon. The study is not yet completed, but the report says that so far there is no strong evidence for an association between herbicides and birth defects.

Two members of an earlier survey of the effects of herbicides in Vietnam, the AAAS study, said last week, however, that the team had not conducted as thorough a study as it could, and suggested that a follow-up is required. Dr John Constable, of Harvard Medical School and Massachusetts General Hospital, suggested that the academy team had failed to look more closely at the figures from the Saigon Children's Hospital, particularly in the years following the AAAS study. "They reexamined our figures", he said, "but they have not attempted to add figures of their own".

The AAAS study pointed out that incidence of cleft lip, relative to other birth defects in the records of the Saigon Children's hospital, showed a marked increase in the years 1966-1968, the years of heaviest herbicide spraying. Although the AAAS team, which was led by Meselson, was careful to say that this effect was not necessarily correlated with herbicide spraying, the figures at least bear close scrutiny. The fact that the academy team had not looked at data from the hospital in the years after 1970 is, according to Meselson, "disappointing".

If there is a teratogenic effect from herbicide spraying, it is likely to have come from the TCDD contamination in the agent orange. TCDD—which is often more familiarly referred to as dioxin—is an extremely potent teratogen in mice, and when it was discovered in 1970 that it is always present in 2,4,5-T, there was great consternation about the risks to Vietnamese people exposed to agent orange. The chemical is such a potent teratogen that it is feared that amounts too small to be detected may prove to be harmful if they get into the food supply.

Last year, however, Meselson and his colleague Robert T. Baughman, reported an extremely sensitive method for detecting TCDD, which they used to analyse fish samples taken from South Vietnam in 1970. They found levels of the chemical ranging from 0.0007 parts per million to 0.008 parts per million. They are now in the process of analysing fish and shrimp samples taken last year, and preliminary results indicate that there is still some contamination with TCDD. The academy committee considered

these findings to be "a matter which warrants further attention". Use of Meselson's method for detecting dioxin in the food supply would be an ideal basis for selecting populations to study for birth defects.

Although the academy's study will clearly spark off a considerable debate about the conclusions that have been drawn, there is no doubt that it has added a huge amount of information on one of the most contentious issues to arise from the Vietnam war. In fact the report published last week was only a summary of the data (even though it ran to 400 pages), and the academy intends to publish later this year all the documentation gathered together during the three years that the report was in preparation.

It remains to be seen whether this wealth of information is sufficient to build up a strong enough case to convince the Administration that herbicides should be banned from use in war. The United States has yet to ratify the Geneva protocol of 1925 which outlaws the use in war of chemical weapons. Ratification of the treaty is now being held up because the US Senate insists that the protocol covers herbicides and tear gases, while the Administration insists that it doesn't. Secretary of State Henry Kissinger informed the Senate Foreign Relations Committee last year that the Administration's position is under review. The academy study is likely to play a considerable role in that review.

Unfilled places

MORE than six thousand university places, mainly in science, engineering and technology subjects, went unfilled in Britain at the beginning of the current academic year. The figures are contained in the eleventh report of the Universities Central Council on Admissions (UCCA), the body which handles the applications of most intending students in Britain. According to UCCA, the British universities expected to receive 71,875 students in October last year, and in fact only 65,586 places were taken. The shortfall in engineering and technology was 1,991, and in science 3,198. In a foreword to the report, Chairman of the Council Dr Geoffrey Templeman says that although the figures contain an element of estimation, and therefore need to be used with care, the picture they reveal is "not encouraging".

Science policy changes in Canada

David Spurgeon, Ottawa

GOVERNMENT announcements seem able to confuse as often as they can inform, and such was the case with those made recently about the forthcoming changes in federal science bodies in Canada.

The announcements came in the Speech from the Throne at the opening session of Canada's 29th Parliament on February 27, and in press releases issued about the same time by the Ministry of State for Science and Technology (MOSST). Those in the throne speech informed only in the briefest and most general way, while the details offered by the ministry's press releases served mostly to confuse.

The throne speech said: "the Ministry of State for Science and Technology will be developing national science objectives as a basis for exercising enhanced advisory and co-ordinating authority within the government. Two new granting councils will be formed, one for social sciences and humanities and the other for natural sciences".

The ministry's press releases spoke in different terms: "the government plans to strengthen the Ministry of State for Science and Technology in order to ensure a more efficient use of human resources and scientific activities in the pursuit of national goals", said one. "Its (the ministry's) advice will increasingly be taken into account by Cabinet in relation to new science oriented policies . . ." said another, labelled statement by Mme Jeanne Sauve, Minister of State for Science and Technology. "Its impact will also be markedly increased in relation to the assessment of scientific programs and associated expenditures. Close cooperation between the ministry and Treasury Board is developing in these areas and it is the intention to focus attention, not only within the government itself, but also in the public domain on the distribution and extent of expenditures in science".

The question in the minds of some was whether the changes outlined meant that the ministry was finally becoming what they had feared it might become—an all-powerful monolithic instrument of power in Canadian science policy—or whether its statements only gave that impression.

That they did give that impression was confirmed by a quick sampling of early reactions among scientists who had read the press releases or news stories about them. Such reactions ranged from expressions of fear and concern to outright predictions of gloom and doom for the future of science in Canada. The

doomsters saw the moves as a political power play by the fledgling ministry, which they feared would create a super-bureaucracy concerned with administrative efficiency and political expediency rather than the good of science or its judicious application.

But those closer to the federal bureaucracy saw things differently. The realities of the situation meant that such a consolidation of power by the ministry was impossible even if desired, they contended, and the politics of the scientific establishment would assure that there is no cause for apprehension.

The changes outlined clearly in the science ministry's press releases were these:

- The granting function of the National Research Council will be separated from the NRC laboratories and removed to a new council entitled the Natural Science Research Council.

- The granting function for the social sciences and the humanities will be removed from the Canada Council and established in a new granting council to be called the Social Sciences and Humanities Research Council. The Canada Council will continue to remain responsible for support of the arts.

- The Medical Research Council's granting functions will remain undisturbed, but the MRC will be placed into "an appropriate relationship" with a new Inter-council Co-ordinating Committee.

- This new Inter-Council co-ordinating Committee will be established by the government to advise on allocation of funds among the granting councils; ensure coverage by the councils of all recognised disciplines: standardise granting practices; ensure that the needs of inter-disciplinary research are met; and coordinate and advise on council programmes—as well as those of federal government departments—in support of university research.

- The new committee will be chaired by the Secretary of the science ministry and will report to the Minister of State for Science and Technology.

- The science ministry will be involved in development of a science policy framework to provide guidance on scientific activities to all departments and agencies in terms of science objectives, priorities and strategies; the development of science expenditure guidelines for science agencies and the Treasury Board; for collaborating with the Treasury Board in analysing expenditures and programme proposals; and in annually assessing accomplishments of government science activities.

Some saw in these moves control by the ministry of the granting agencies and a pre-eminent position for the ministry in directing the use of fiscal sup-

port for science. This seemed to be confirmed by further announcements that the Science Council of Canada Act would be amended to give the council a more public role, while the ministry would take over its former role as official science advisory body to the federal government.

The announcements were also seen as evidence that the government had taken seriously the recommendations of the Senate Special Committee on Science Policy (the so-called Lamontagne Committee), which in its report No. 3 had said that the science ministry's advisory role was not good enough for its survival, and that "sooner or later MOSST will face a dead end unless it is given more authority . . . the committee believes that the Ministry of State for Science and Technology should become the focus of a central machinery for the concentrated planning and control of government involvement in science and technology".

Where the confusion arose was in certain ambiguities—and certain omissions—in the ministry's press releases. For example, while the press releases referred to the past reporting practices of the NRC and MRC and Canada Council, they avoided saying explicitly where these agencies might report in future, while making it easy for the reader to conclude that this would be, in effect, to the Minister of State for Science and Technology through the new Inter-council Co-ordinating Committee.

In fact the NRC and MRC are both Crown corporations and the Canada Council is a foundation, which report to Parliament through the President of the Treasury Board (NRC), the Minister of National Health and Welfare (MRC), and the Secretary of State (Canada Council). Changes in legislation would be required to change this, and nothing in the throne speech presaged such changes. The heads of the MRC and Canada Council are known to understand that their respective reporting procedures will not be changed. And it seems illogical that a humanities council head would report to the science minister, though the ministry press releases are silent on this point. Nor do they say to whom the new Natural Sciences Research Council will report.

Equally vague are the references to the relationships of the granting councils to each other and to the science ministry as members of the new co-ordinating committee. Even the make-up of the committee is left a mystery: it "will include in its membership, the heads of the granting councils and certain other senior officials to be named later".

Old Ottawa hands see all this as a struggle for power by the science ministry, but a struggle with an extremely uncertain outcome. Concerning the con-

tinued autonomy of the granting councils, which is seen as crucial to the future of science in Canada, a great deal will depend on the way the Inter-council Coordinating Committee works, and this in turn depends greatly on its membership and the effectiveness of its chairman.

The possibility of the ministry using its enhanced position as government science adviser and budgetary watchdog as a path to massive centralisation of power is also seen as unlikely, given the realities of the situation. The science minister is not a member of the Treasury Board and has not the political heft of huge science-related departments like Environment. Nor need the Treasury Board heed the ministry's advice unless it finds it good—it has its own advisers as for other areas, and conducts its own assessments, and will continue to do so. Or so runs the argument.

Whatever the outcome, some observers are wondering what happened to another major recommendation of the Lamontagne report (and one made by other studies): the need for a better linkage between government R&D and industry. None of the re-organisational changes seem bent toward this goal—and in fact the splitting of NRC's laboratories from the granting council (which provided such a link) seems actively to work against it. The NRC laboratories, it seems, will be isolated.

Velikovsky in the open

Miranda Robertson, San Francisco

THE scientific session on the heterodox theories of Immanuel Velikovsky at the meeting of the American Association for the Advancement of Science (AAAS) in San Francisco on February 25 was the first and probably the most serious of four symposia to be held this year on various issues raised by Velikovsky's ideas and his attempts to propagate them. The organisers of the AAAS symposium adopted the stance that Velikovsky has offered a 'challenge to science'. Science, in the persons of Dr P. J. Huber (Swiss Federal Institute of Technology, Zürich), Dr J. D. Mulholland (University of Texas) and Dr C. Sagan (Cornell University) rose to meet it.

The only remarkable thing about the resulting session was the amount of time and energy Drs Huber, Mulholland and Sagan had been prepared to put into it. There seems little danger that any real scientist or scholar would be tempted to take Velikovsky's ideas seriously. Best-selling books are usually fiction, and Velikovsky's *Worlds in Collision*—

published in 1950 by Doubleday after the Macmillan Co., showing more academic integrity than business acumen, had passed it on to them—is plainly no exception. Velikovsky himself is by birth a Russian Jew trained in medicine and psychiatry.

Ostensibly, the fundamental issue at the symposium was Velikovsky's version of the birth of Venus. His story is that the planet originated as a comet torn out of Jupiter about 1500 BC, when it went into an eccentric orbit in which it narrowly missed the Earth a number of times and disrupted the orbit of Mars before settling down about 800 BC in its present position in the solar system. All this is documented in the Bible (the seven plagues of Egypt, the Israelites' manna from Heaven and various other miraculous phenomena were the consequences of cosmic catastrophe) and in some ancient Egyptian writings.

Huber was brought in to deal with Velikovsky's Egyptology and Assyriology, Mulholland to tackle his celestial mechanics, and Sagan to do battle energetically on all available fronts at once. Velikovsky, however, turned out to be too easy to discredit. He muddles his myths, confusing Athene and Aphrodite; and his chemistry, confusing carbohydrates with hydrocarbons—so that Venus at once bestows upon the Earth supplies of petroleum (hydrocarbon) and manna for the children of Israel (carbohydrate) from its atmospheric clouds, now known in any case to consist of sulphuric acid. The spin resonance of the Earth with Mars and Venus can only be explained by the stability of all three planets in orbit in the solar system for billions of years. Cuneiform texts reliably dated well before 1500 BC refer to a Venus behaving recognisably as Venus behaves today.

Velikovsky replied to his critics with rhetoric not reason. The issue, in short, was not scientific but sociological. Indeed it was stated at the beginning of the session by Dr N. Storer, who is a sociologist at the City University of New York: in principle, the progress of science depends on the patient and impartial evaluation by the scientific community of all new ideas and work. In practice, no-one has time to examine the arguments of every heterodox claimant to new insights and it is surprisingly easy to be certain in the majority of cases that one is not withholding recognition from a second Einstein.

Velikovsky, however, claims not merely to have been ignored but to have been unfairly attacked by the scientific establishment. This was the real burden of his challenge, and the attempt to deal fairly and seriously with his thesis succeeded in illustrating exactly what makes scientists lose patience with the majority of far-reaching new theories. Generally,

they are so loosely stated as to present inadequate premises for logical or quantitative reasoning. This point was emphasised by Dr Sagan, both explicitly and implicitly in his struggle to find the substance of Dr Velikovsky's theories.

For Sagan—himself no mean propagator of wild theories, as might be inferred from his editorship of the journal *Icarus*—the professed object of the apparently unedifying exercise in San Francisco was the demonstration of the essence of scientific method, and what happens when its criteria are applied to fantasies such as Velikovsky's.

Actually, like the symposium on unidentified flying objects held by the AAAS five years ago, it was an exercise in setting the record straight. The sensational speculations are made available to everyone by the popular press, but where are people to find the sober evaluations? Sagan has suggested that this should be an occasional but regular function of organisations such as the AAAS. In many ways this seems a more plausible justification for unwieldy annual science meetings than the pretence that they do anything to further the cause of science. Furthermore, the subject matter for the meetings would thus be selected by an entirely democratic process, since the only criterion for including a subject in the agenda for discussion would be the amount of public interest it had aroused. Whether the public actually wants to be disabused of sensationalist notions is, of course, another matter.

Seeing double

ANOTHER curious piece of programming by the BBC on Monday March 4 produced "Horizon" on BBC2 telling us that "The Future Goes Boom!" immediately followed by "What on Earth . . . are we doing?" on BBC1 in which Dr David Bellamy told us that we might just struggle along if we all tighten our belts. A more fruitful approach might have been to confront Professor Hermann Kahn, the prophet of boom, with Bellamy to thrash out the issues. As things stand, the protagonists are about even; both programmes opened with film of an Apollo launch, but the print used by the "Horizon" team was much sharper and in better colour. To balance this, Bellamy's subsequent down-to-Earth approach made more sense than Kahn's frenetic globe trotting while calculating, apparently on the back of the proverbial envelope, just how many people are likely to be killed in the forthcoming nuclear war. But a solid 2½ hours of proselytisation with no lucid discussion or pause for thought probably turned most viewers completely off, if they did not first turn it off.

news and views

When the lunar crust formed

THREE distinct aspects of lunar history have been dated from age determinations of rocks from the Moon. The basaltic lavas filling the visited maria sites have ranged in age from 3,100 to 3,900 million years. These lavas were produced by melting or partial melting of rock in the interior of the Moon by heat generated by the radioactive decay of uranium and thorium. The implied absence of younger rocks over a substantial part of the lunar surface is explained in part by the concentration of radioactive heat sources in the outer regions of the Moon which, coupled with the smaller size of the Moon, makes for a more efficient heat loss than is the case for the Earth. Internal motions within the lunar interior have been unable to disrupt the relatively thick crust and in consequence there have been no large scale horizontal movements of the crust to assist the process of continuing lava extrusion, again in contrast to the Earth. In general the lavas have been extruded into large, impact-generated basins, possibly along lines of weakness related to the impact.

An earlier episode of lunar history is represented by the dating of the complex fragmental rocks from the various highland sites. The ^{40}Ar - ^{39}Ar and Rb-Sr (mineral isochron) ages of highland rocks are, with a few exceptions, confined to a narrow time interval from 3,900 to 4,000 million years, indicating that the rocks were last at elevated temperatures at this time rather than that this is when their individual components crystallised. The ages have been taken by several authors to indicate the times when a number of the large impact basins were formed but there is no consensus as to how many basin-forming events are represented in the ages, and estimates vary between two (Orientale and Imbrium) and six (Orientale, Imbrium, Crisium, Nectaris, Humorum, Serenitatis). All authors are agreed that the first 500 million years of lunar history has been obscured by the intense bombardment of the highlands.

Evidence for the earliest period of lunar history has so far come from a comparison of the isotopic composition of Sr and Pb in suites of rocks from the various sites, in the lunar soil, and in one particularly exotic rock, 12013 from Apollo 12. All of these rocks have been subjected to reheating or melting at or more recently than 4,000 million years ago, but it is possible to draw inferences about the isotopic composition of the precursor material and specifically to show that regions of distinct chemical composition (differing Rb/Sr and U/Pb ratios) existed as early as 4,500 million years ago. The physical implication of this is that melting and the separation of chemically distinct rock types must have begun as early as 4,500 million years ago—shortly after the Solar System formed. The heat source responsible for this early melting is not clear although the gravitational potential energy released in accretion would be adequate if accretion were sufficiently rapid.

In the article on page 199 of this issue of *Nature*, Jessberger, Huneke and Wasserburg have used a third technique, the ^{40}Ar - ^{39}Ar method, to find evidence of relict crustal material in a 4,000 million-year-old breccia. The ^{40}Ar - ^{39}Ar method is a modified form of K-Ar dating first applied at the University of Sheffield to the dating of lunar samples from Apollo 11. It is a neutron activation technique which con-

verts some of the potassium in the rock to ^{39}Ar , and the age can be determined from the ($^{40}\text{Ar}/^{39}\text{Ar}$) ratio in the gas extracted by heating the sample at a succession of increasing temperatures. The method is in principle able to detect variations in the ($^{40}\text{Ar}/\text{K}$) ratio which could result from partial argon loss in a sample with a complex thermal history and in suitable circumstances to unravel that history.

Jessberger, Huneke and Wasserburg have applied the technique to separated minerals from a highland breccia which previously had been shown by Rb-Sr measurements to have been heated 3,980 million years ago but to temperatures insufficient to homogenise the Sr isotopic composition. The ($^{40}\text{Ar}/^{39}\text{Ar}$) ratios measured in a heating experiment of 30 to 50 steps correspond for the most part to this age of 3,980 million years, apart from small variations which seem to be an artefact of the technique, possibly related to the recoil of ^{39}Ar during neutron activation. In the highest temperature extractions from a sample of the plagioclase which showed the anomalous Sr isotope composition, however, the ($^{40}\text{Ar}/^{39}\text{Ar}$) ratio increased to a value corresponding to 4,500 million years. This would seem to indicate that when the breccia went through the heating episode at 3,980 million years most of the preexisting ^{40}Ar was driven off, except in some very retentive crystal sites in the plagioclase, and that the argon in these sites is retaining a memory of when the plagioclase originally crystallised as part of the early lunar crust, 4,500 million years ago. Thus all of the radioactive clocks now provide independent evidence that the crust of the Moon was beginning to form a very short time after the Solar System itself formed.

G.T.

From genes to behaviour

THERE is many a potential intermediary process twixt the gene and the behaviour it affects. This has been a general problem facing behaviour geneticists as they search for clues on the mechanisms of gene action. Sometimes the answer has been boringly obvious—the mutant mouse which builds nests larger than normal turns out to have thinner fur, and so on. But a number of the older studies on genes affecting nest cleaning behaviour of honeybees (Rothenbuhler, *Am. Zool.*, **4**, 111–123; 1964) or courtship displays of *Drosophila* (Bastock, *Evolution*, **10**, 421–439; 1956), for example, revealed effects which could reasonably be explained only by action on the central nervous system. There is, of course, no shortage of neural elements as potential sites for primary gene action, and for complex behaviour patterns it is difficult to take the story further.

The past few years have seen some important developments in behaviour genetics, relying on the identification of behaviourally-effective mutants induced in *Drosophila* and nematodes. The philosophy behind such work contends that behaviour genetics must be built from the bottom upwards, concentrating first on the simple behavioural repertoire of organisms with simple nervous systems.

Brenner and his group at Cambridge have progressed further than most with their nematode *Caenorhabditis elegans*, which has a nervous system of only a few hundred elements. A series of mutants which affect the worm's locomotion have been identified and some of these produce detectable and pre-

cisely regular defects in neural interconnectivity. Some of these defects are confined to one or two neurones whereas others affect every one of a series along the worm's body.

The regularity of nematode structure allows a finer-scale of analysis than is yet possible with *Drosophila*. Hotta and Benzer (*Nature*, **240**, 527-535; 1972) have used an elegant technique involving genetic mosaics to localise the site of action in their *Drosophila* behavioural mutants. They can definitely ascribe some of these to the brain alone, but so far only Ikeda and Kaplan (*Proc. natn. Acad. Sci. U.S.A.*, **66**, 765-772; **67**, 1480-1487; 1970) have pinned down a neural site precisely. One of their *hyperkinetic* mutants, which shows abnormal leg movements under ether anaesthesia, has modified spontaneous activity in specific motoneurons lying laterally in the thoracic complex. Not only have Ikeda and Kaplan been able to isolate these neurones from inputs and show that they retain the abnormal pattern, but they have used the most perfect control for systematic effects—a left/right mosaic in which the normal left half of the body can be compared with its mutant right.

On the sensory side Benzer's group, and others, have isolated several mutants which affect the electroretinogram response of the *Drosophila* eye. Most of these were picked up by screening the responses of large numbers of flies to various visual stimuli. Eyes are complex and mutants affecting the chemical senses have the advantage of a much simpler neural substrate. Ward (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 817-821; 1973) has already described some genes affecting the chemotactic responses of *Caenorhabditis*. Kikuchi (*Nature*, **243**, 36-38; 1973) found a dominant in *Drosophila* which caused flies to be attracted by chemicals which repelled normal individuals. Now in this current issue (page 243) Isono and Kikuchi describe another autosomal recessive with a specific effect on the chemosensory hairs of the labellum (and presumably also those on the tarsi) which respond to sugars. In particular the mutation greatly reduces the response to glucose. Such a mutant should prove useful for the identification of receptor sites for sugars at the hair tip.

Slowly a catalogue of behavioural mutants is being built up. The bricks are few and the wall to be constructed is of enormous dimensions, but soon one may be able to detect some pattern in the brick laying. Nevertheless, those working with complex behaviour will have to wait some time for explanations of behavioural potential in genetic terms.

A.M.

Capping related to type of cell

TREATMENT of lymphocytes at 37° C with low concentrations of reagents such as concanavalin A or anti-immunoglobulin antibody leads to movement of the binding sites into large patches known as 'caps'. It now seems that the situation established previously is by no means a biologically homogeneous event. Stackpole, Jacobson and Landis show convincingly on page 232 of this issue of *Nature* that the exact location on the cell surface at which a cap forms depends in a critical and as yet undetermined manner on the type of cell and to some extent on the particular surface molecules being capped. Caps can form over or opposite to the Golgi region of the cell. Pinocytotic vesicles are seen only when the cap is formed over the Golgi region. Furthermore the new results may help clarify several puzzling and conflicting observations of the effects of drugs such as cytochalasin B on cap formation.

Caps are formed in the classical way by first exposing mouse thymus or spleen cells to specific mouse alloantibody directed against a particular surface membrane antigen and this is followed by cross linking with anti-mouse immuno-

Are supernovae disruptive?

It is arguable that all supernova explosions occur in binary systems, as a result of mass transfer between close companions. It is also arguable that all pulsars are created in supernova explosions. So the lack of any evidence to show that any pulsar is a member of a binary system looked at first sight embarrassing, and led in turn to the suggestion that supernovae always disrupt the binaries which give them birth. But the more recent discovery of X-ray 'pulsars' which are members of binary systems has encouraged a retreat from this extreme position; on page 208 of this issue of *Nature* Sutantyo shows that present theories of stellar evolution do indeed suggest that supernovae occurring in close binaries need not be disruptive in all cases.

It is interesting to speculate that this work may form the basis of a theoretical understanding of why some binaries should explode to produce variable X-ray sources, whereas others are turned into pulsars. As Sutantyo points out, the known X-ray binaries each contain an OB supergiant with mass some tens of solar masses, with a companion of around 0.5 to 5.0 $M_{\odot}$; the large mass ratio suggests that the explosion occurred before mass transfer. In that case, the less massive star is probably the one involved in the explosion, and all such binaries with periods larger than 5 d remain bound after the explosion. That is more than 50% of all such systems.

Perhaps, as Sutantyo suggests, the only reason that radio pulses are not visible from such binaries is that the radio emission is absorbed and smeared out by the gas flows between the two stars. Any remnant which is left on its own by a disruptive explosion cannot remain an X-ray source for long, since this energetic emission requires a constant input of gravitational energy released by mass transfer between the components, according to the best theories. So such unattached remnants would inevitably be seen as radio pulsars, if they were seen at all.

And that, of course, raises the question of whether such sources are merely the minority cases from the explosions considered by Sutantyo (which seems unlikely in view of the number of pulsars known) or whether other kinds of binary system are indeed more often disrupted than not as a result of supernova explosions. Certainly there is more than one kind of supernova; do the different kinds produce different offspring?

J.G.

globulin antibody. Stackpole *et al.* used hybrid anti-mouse IgG/anti-ferritin antibodies that are revealed on the cell surface by adding ferritin. In this way it was found that a very high proportion of spleen cells display a cap of ferritin and this indicates, of course, a similar distribution of the underlying cell surface antigen. Almost without exception the caps were located over the Golgi elements of the cell when surface immunoglobulin molecules or concanavalin A receptor sites were capped. By contrast capping of H-2 antigens on mouse spleen cells is not polarised but patches form over and opposite to the Golgi region. A clear difference is seen with mouse thymus cells compared with spleen cells: in the first place Thy-1 and TL alloantigens capped predominantly over the Golgi region in spleen cells, whereas the Thy-1 alloantigen of thymus cells, for instance, is moved into the opposite pole of the cell.

It was shown previously by several groups that cap formation is often accompanied by pinocytosis of labelled membrane. After a relatively short period at 37° C cells may have much of their ferritin label within the cytoplasm and attempts to relabel the surface with reagents specific for the internalised receptors give negative results. It seems that pinocytosis takes place only in the special case in which caps form in the vicinity of the Golgi apparatus. Thus, caps of Thy-1, surface immunoglobulin or concanavalin A binding sites on mouse spleen cells are extensively internalised. In thymus cells very little pinocytosis of labelled surface markers takes place.

Stackpole *et al.* can distinguish further the two types of capping by the effects of certain inhibitors. General inhibitors such as sodium azide (and probably dinitrophenol) inhibit capping of all antigens both in spleen and thymus cells, but cytocholasin B has an effect only on the movement of antigens towards

the pole of the cell immediately adjacent to the Golgi apparatus. The drug therefore has almost no effect on capping of H-2 antigens on spleen cells, and redistribution of thymus cell surface markers is also not inhibited. In agreement with earlier studies, reagents affecting cellular microtubules, such as colchicine, vinblastine and colcemid, do not inhibit either type of capping. Indeed according to Berlin *et al.* (*Nature*, **247**, 45–46; 1974) colchicine in certain conditions even facilitates capping of concanavalin A receptors. The effect of cytocholasin B, however, does suggest that at least one form of capping is actively dependent on intracellular contractile elements.

The way in which the direction of capping is controlled for a particular surface marker—for example H-2 antigens in spleen cells—remains to be determined. Clearly, the situation is very complex and many cellular processes providing multiple target sites for drugs are probably involved. R.C.H.

Translational repression

from a Correspondent

It is well known that the coat protein of group I and III RNA bacteriophages can act as repressors of the synthesis of the replicase by binding to their homologous RNA. Three reports in *Nature* this week (see pages 204, 223 and 225) by Steitz and two groups of collaborators shed fascinating light on the details of this process. Two of the papers are concerned with the secondary structure of the fifty-nine nucleotide-long segment of R17 RNA which is protected against T₁ nuclease attack by the coat protein and which has been sequenced. It contains the end of the coat protein gene including the tandem terminator and extends through the intergenic region to the initiator region of the following replicase gene. Inspection of this sequence has suggested the presence of two helical regions, A and B; the first involves the coat protein terminator and the second the replicase initiator region.

Gralla, Steitz and Crothers from Yale have studied the melting behaviour of this fragment and demonstrate that differential melting curves exhibit two sharp peaks indicative of two substantial helical regions. Analysis of the details of these peaks allows estimations of the T_m and enthalpy of melting of these two helices. These estimations are in good agreement with the values calculated for the two helical regions A and B by methods worked out by the same group and assuming that helix B melts at the lower temperature.

This assignment of the lower melting peak to helix B is confirmed by the

work of Hilbers, Shulman, Yamane and Steitz from the Bell Telephone Laboratories who have studied the nuclear magnetic resonance (NMR) spectrum corresponding to the ring NH protons which are involved in the formation of A-U or G-C pairs. Their data suggest that at 26° C there are sixteen base pairs of which eight are no longer apparent at 40° C. A detailed analysis of the NMR spectra in terms of sequence-dependent ring current shifts suggests that the eight base pairs that are lost on increasing the temperature are derived from helix B. The disappearance of the signals corresponding to these base pairs does not necessarily imply that the helix is melted completely by 40° C but only that the mean lifetime of a base pair is less than about 5 ms. Indeed the data of Gralla *et al.* suggest that both helices are essentially intact for most of the time at 25° C in the presence of moderate concentrations of cations.

Gralla *et al.* went on to study the kinetics of the melting processes by temperature jump techniques. These measurements give time constants for these processes and also their amplitudes. As the temperature was increased from 25° C, a rapid process was observed with an amplitude that increased to a maximum at the lower T_m . Thereafter this amplitude decreased and was replaced by a slower process with an amplitude that increased to a maximum at the higher T_m . Clearly these two processes reflect the independent melting of two helical regions.

In further studies, Gralla *et al.* found by sedimentation equilibrium measurements that the molecular weight of a complex of the coat protein and this fragment reached a plateau value for a 1:1 mixture. Melting studies of this 1:1 complex revealed that although the T_m of neither helical region was altered by binding of the coat protein, the rate of melting of the lower melting helix B was reduced by a factor of 100 while that of helix A was unchanged. After considering various alternative hypotheses, these authors favour an interpretation that involves the binding of helix B to the coat protein and some irreversible process, perhaps involving the denaturation of the protein at the T_m of the helix.

That the coat protein recognises the B helix is further confirmed by the work of Steitz in the third paper who found that the coat protein will also bind to a twenty-nine nucleotide-long portion of the larger fragment which incorporates the B helix but not the A. Furthermore, an oligonucleotide from the turn of the B helix will not bind efficiently and this suggests that the B helix itself is recognised by the coat protein. Steitz points out that the sequestration of the initiator region in the B helix could account for the inability of *Escherichia coli* ribosomes to recognise the replicase initiator region.

It is emphasised by the authors of these papers that these helical regions may not exist in the intact R17 RNA at all times, especially when ribosomes do succeed in recognising the replicase initiator region.

Ice cover and global weather shift

by our Cosmology Correspondent

"SNOW and pack-ice cover in the northern hemisphere formed earlier in the year and covered a larger area in the past 3 years than it did 7 years ago, when systematic satellite mapping began. This shift, in all likelihood, has produced a significant change in the hemispheric heat balance. The difference was most pronounced in the fall and was especially large in 1971. The anomalous global weather patterns of 1972 and 1973 may be the result of these developments."

That is the conclusion reached by G. J. and H. J. Kukla after studying weekly maps supplied by the US National Oceanic and Atmospheric Administration (NOAA) and based on photographs obtained by meteorological satellites. All snow and ice fields lasting more than 5 days are recorded on these photographs. The dramatic implication of this work is that with a better understanding of how the Earth's atmosphere

works, the changes which have caused such trouble in the northern hemisphere recently (and indeed those which are causing trouble in Australia now) might have been predicted in time for effective action to be taken.

As Kukla and Kukla point out (*Science*, **183**, 709; 1974), the location and duration of snow and pack ice fields are the most important seasonal factors which affect the Earth's heat balance. Because of the heat required for melting snow and ice, peak air temperatures in summer at mid-latitudes are delayed after the peak insolation. In the northern hemisphere, permanent ice covers some 10×10^6 km², and seasonal ice and snow covers a further 50×10^6 km²; in the south, the corresponding figures are 14×10^6 km² and 20×10^6 km². The 'temporary' cover in the north is greater, of course, because of the presence of continents on which the snow can lie. Satellite mapping provides the only reliable means of monitoring the changing ice patterns over a whole hemisphere.

These studies are facilitated by dividing the year into four snow cover (SC) seasons. SC summer is the part of the year when less than 15×10^6 km² of

the northern hemisphere are covered; SC winter the period when more than 55×10^6 km² are covered. During the past 7 yr, SC winter and SC summer have been about the same length, averaging 80 to 90 d. But SC spring was, on average, about 7 weeks longer than SC fall. And the date on which SC fall and winter started were earlier in each of the successive years 1971, 1972 and 1973. In 1971, the change was dramatic, with SC fall starting 3 weeks earlier than in any of the 4 preceding years.

During the same period, there was a gradual increase in snow and ice cover found during the October/November period. For the entire 7 yr of this study, the average annual coverage was 34.9×10^6 km²; in 1971 alone this increased by 12% and since 1971 the average has fluctuated about a new mean of 36.9×10^6 km². On November 1, 1973, the cover was 36.7×10^6 km². So the period since 1967 divides naturally into two distinctly different sections.

Kukla and Kukla discuss at some length the possible sources of error in their study, and argue a convincing case that the effect they report is real. "There is no doubt", they say, "that the surface heat exchange in 1971 must have dropped

markedly". It is still too early to say definitely that this caused the change in global circulation which has, in recent months, been the subject of a great deal of discussion both in these pages and elsewhere; but it may well be significant that the changes in ice cover occurred before these changes in circulation became apparent. It seems plausible, at least, to argue that the observed changes in mean temperature and other parameters during the past 30 yr eventually produced the increased snow cover which has in some way disturbed the atmospheric circulation.

Superfluid ³He confirmed

from our
Condensed Matter Correspondent

THE report in a recent issue of *Physical Review Letters* (**32**, 141; 1974) by Kojima, Paulson and Wheatley of the University of California at San Diego that they have observed the propagation of fourth sound in the two newly discovered phases of liquid ³He amounts to the first unequivocal evidence that both of these new phases are superfluids.

It has long been known that liquid ⁴He is superfluid below its transition temperature at about 2 K but, until 1972, it was part of the conventional wisdom that the other isotope, ³He, was not a superfluid. This was mainly because of the theoretical impossibility that liquid ³He, whose atoms contain an odd number of fundamental particles and are called fermions, could become superfluid in the same manner as liquid ⁴He by undergoing a Bose-Einstein condensation; this phenomenon can only occur in assemblies whose components, bosons, contain an even number of fundamental particles. It was realised, however, that the electrons (also fermions) in a superconductor, at that time the only other known superfluid, acquired their superfluidity in a somewhat different way from ⁴He; and it had been suggested that a transition analogous to the superconducting transition in metals might occur in liquid ³He at a sufficiently low temperature, leading to some sort of superfluid state.

The first indication that this suggestion might indeed have been correct came some fifteen years later when Osheroff, Richardson and Lee of Cornell University discovered that two quite separate phase transitions occurred in a cell containing a mixture of liquid and solid ³He when it was cooled under pressure below 3 mK (*Phys. Rev. Lett.*, **28**, 885; 1972). In subsequent experiments, Alvesalo, Anufriyev, Collan, Lounasmaa and Wennerström of the Technical University at Helsinki used

Synthesis *in vitro* of silkworm ecdysone

from our Insect Physiology Correspondent

TWELVE months ago some of the queries that have arisen concerning the site of synthesis of ecdysone in insects were discussed (see *Nature*, **242**, 86; 1973). Briefly, the problem has been that, in spite of the conclusive experimental evidence (provided originally by Fukuda) that the moulting factor of insects is produced by the prothoracic glands, it has not proved possible to get convincing proof that the product ecdysone as isolated by Karlson and Butenandt is synthesised by these glands. Only the most minute amounts of ecdysone from the prothoracic glands have been extracted, and glands maintained *in vitro* produced only insignificant amounts of the hormone.

A group of Japanese workers led by Chino (*Science*, **183**, 529; 1974) have now reinvestigated this problem and have found, as earlier workers had done, that the isolated prothoracic glands of the silkworm *Bombyx* maintained in the culture media of Grace or of Wyatt produced only minute amounts of ecdysone (less than 5 ng per pair of glands). Unchanged haemolymph blackens and is an unsatisfactory medium; but by isolating the haemolymph proteins on a Sephadex column and suspending these in Wyatt's culture medium and saline Chino and his colleagues attained a far more satisfactory "haemolymph medium". Arguing that the rich tracheal

supply of the glands indicated a high oxygen requirement for synthesis, they exposed the gland pairs, each pair in a single drop culture, to oxygen instead of air. By these combined procedures the hormone produced per gland pair increased in amount from 5 ng to 120 ng.

It is well known that the insect requires cholesterol for ecdysone synthesis. The authors therefore concluded that the lipoproteins of the haemolymph are likely to be the essential component so they then purified the two chief lipoproteins I and II from the haemolymph and prepared a culture medium by addition of these to Wyatt's medium—and this proved even more effective than the haemolymph medium. The hormone synthesised by the glands is at once liberated to the medium; in agreement with earlier observations, almost no ecdysone can be extracted from the glands themselves. Thin layer and gas chromatography established that the hormone produced by the glands is solely α ecdysone; as had already been suspected the more active and more polar product, β ecdysone must be formed from α ecdysone elsewhere in the body. Chino *et al.* report that another research team in the laboratory of Gilbert, working independently, has obtained similar results with the prothoracic glands of the tobacco hornworm *Manduca sexta* (*Proc. natn. Acad. Sci. U.S.A.*, in the press).

a vibrating wire viscometer to show that the viscosity of the liquid fell rapidly with decreasing temperature below the higher temperature transition in the so-called A phase of the liquid, and even more rapidly in the B phase below the second transition (*Phys. Rev. Lett.*, **30**, 962; 1973). This result strongly suggested the onset of superfluidity but the evidence was not conclusive in that the experiment did not demonstrate unambiguously the presence of any fluid with zero viscosity.

Fourth sound is a phenomenon well known in the case of liquid ^4He , which can be regarded as an homogeneous mixture of normal and superfluid components; it is a wave motion which can occur when the liquid is absorbed into a tightly packed powder. In these conditions the normal fluid component, which has a finite viscosity, is effectively immobilised but the superfluid component, with zero viscosity, is still entirely free to move. The pressure wave which can thus be transmitted through superfluid in the interstices of a tightly packed powder is known as fourth sound; and the densities of the normal fluid ρ_n and of the superfluid ρ_s can be deduced from measurements of its velocity. This wave mode can exist only if some superfluid is present, so it was natural to search for a possible fourth sound mode in each of the new phases of liquid ^3He .

The experimental cell used by the San Diego group was constructed from an epoxy resin and was tightly packed with finely ground crystals of the paramagnetic salt cerium magnesium nitrate (CMN). Pressure transducers were positioned at either end of the cell so that fourth sound, if it existed, could be both excited and detected. The function of the CMN was threefold: its adiabatic demagnetisation enabled the liquid ^3He trapped in the interstices to be refrigerated to the required temperature; it acted as the superleak in which the fourth sound could be excited; and the temperature could be deduced from measurements of its paramagnetic susceptibility. In the event it was found that fourth sound could indeed be propagated, not just in one, but in both of the new phases, proving beyond doubt that both are superfluids.

The velocity measurements were used to derive values of ρ_s , which turned out to be surprisingly low: much lower, in fact, than may be deduced from the Helsinki viscosity experiment. The reasons for this apparent inconsistency are not yet clear. The San Diego group admit, however, that their values of ρ_s may perhaps be strictly relevant only to liquid trapped in fine pores, since it is known that for ^4He under similar conditions ρ_s is somewhat smaller than in the bulk liquid. Be this as it

may, the group's experiment has now confirmed what many physicists had come to believe would probably turn out to be the case: that, with the discovery of the new phases of ^3He , the number of known superfluids in nature has been doubled.

Resolving conflicts by time sharing

from our Animal Behaviour Correspondent

MUCH is now understood about the control of drinking in thirsty animals and feeding in hungry animals, but relatively little attempt has been made to understand how animals behave if they are made both hungry and thirsty and given access to both food and water. Problems of such motivational 'conflicts' are of great interest in finding out how behaviour in general is organised as most animals are probably in some form of conflict for much of their lives; for example, whether to attack a rival or flee, to feed or incubate eggs. McFarland and Lloyd (*Q. Jl. exp. Psychol.*, **25**, 48-61; 1973) now suggest that at least in the case of feeding-drinking conflicts some animals may resolve their conflicts by operating in a manner analogous to 'time of sharing' in computers.

It had previously been found (McFarland, *Rev. Comportement Anim.*, **4**, 64-73; 1970) that in doves, the normal course of feeding or drinking is characterised by pauses during which other types of behaviour occur. For example, a hungry dove would pause at various times during feeding and if water were present, it would drink during these pauses. Interestingly, the times when the pauses in feeding occurred were not affected by either the presence of external drinking stimuli or, within limits, on how thirsty the doves were. On this basis, the drinking was said to occur by 'disinhibition': it was allowed to occur during preprogrammed breaks in the dominant behaviour of feeding.

McFarland and Lloyd define a dominant behaviour as one which is always resumed following an interruption and a subdominant behaviour as one which is resumed only if the duration of the interruption is less than that of the preprogrammed break in an alternative (dominant) behaviour. Primarily hungry animals interrupted during feeding return to feeding, and primarily thirsty animals interrupted during drinking return to drinking, so that by suitable manipulation of food and water deprivation schedules, either feeding or drinking could be made to be the dominant motivation. Their new evidence suggests that not only does the dominant system determine when subdominant behaviours occur, it also determines how long the

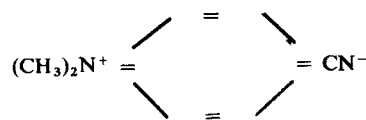
behaviour continues. They found that when a primarily hungry animal is interrupted while drinking or a primarily thirsty animal while feeding, the same behaviour is resumed after a short interruption, but changes to the alternative behaviour after a long interruption. In other words, if a subdominant behaviour is interrupted for a period which is longer than the pre-programmed break in the dominant behaviour, when the interruption is over it will be time for the animal to return to the dominant behaviour. An interruption which does not last longer than the allowable break in dominant behaviour will be followed by resumption of subdominant behaviour.

McFarland and Lloyd argue that when, as in the present case, the occurrence of an activity is determined by another motivational system both in terms of frequency and duration, then this should be called 'time sharing', by analogy with similar systems used on computers to overcome a similar problem—namely, to deal efficiently with simultaneous demands from several sources on limited parts of the machine.

Solvent reorganisation around a 'giant dipole'

from our Chemical Physics Correspondent

IT is difficult to find a new corner of science to develop these days, even a small corner can be elusive. This is, however, what Struve, Rentzepis and Jortner seem to have found in their paper on the kinetics of solvation (*J. chem. Phys.*, **59**, 5014; 1973). The thermodynamics of solvation of ions and molecules is a well studied subject but for kinetic information one must find a non-equilibrium, non-solvated species and then follow the development of solvation in the next few nanoseconds or less.



Jortner and his colleagues have solved this problem by studying the process after electronic excitation in *p*-dimethylaminobenzonitrile. In its ground state such a molecule is only weakly solvated, but its second singlet excited state corresponds to a quinoid formula (see figure) with its very large charge separation and a dipole moment of $77 \times 10^{-30} \text{ C m}$ (23 debye). Each end of the molecule will be heavily solvated, at least in polar solvents whose molecules will align themselves around the separated charges. At the instant of electronic excitation the molecule finds itself essentially unsolvated and it is the rate of subsequent solvation which

is of interest. The production of the species with non-equilibrium solvation is just the problem of exciting molecules to the quinoid state and this is accomplished with a laser flash at $37,600\text{ cm}^{-1}$ (265 nm) obtained as the fourth harmonic of a Nd³⁺ glass laser of duration about 5 ps.

The second half of the problem is to determine whether or not, at a subsequent moment of time, the quinoid state is yet solvated. This is answered by measuring the emission spectrum. If the solvation remains weak, as it will be in non-polar solvents such as methyl cyclohexane, the excited molecule loses its energy, first, by radiationless conversion to the lower excited singlet state and finally by fluorescence from this state at $28,600\text{ cm}^{-1}$ (350 nm). This process requires 20 ps and the fluorescence was detected photographically at the appropriate spectroscopic position. If the solvent consists of polar molecules, such as ethyl alcohol, the quinoid excited state is stabilised by solvation and is detectable as a new fluorescence emitter at $21,300\text{ cm}^{-1}$ (470 nm). Such fluorescence requires 50 ps to build up to maximum intensity and it is concluded that this delay corresponds to the time required for the polar solvent molecules to orient themselves around the giant dipole excited molecule. This seems a reasonable order of magnitude but the first experiments are not very precise; however, the experimental difficulties with delay paths, light shutters and so on are considerable. After all, in 50 ps light travels less than 2 cm.

New interpretation of Keweenawan rift

from our Geomagnetism Correspondent

THE 'midcontinent gravity high' of central North America is a linear belt of positive Bouguer anomalies which stretches about 1,300 km in a north-east-south-west direction from Lake Superior to Kansas. In numerical terms, the positive anomalies reach values as high as +60 mgal and for much of their length are flanked by parallel gravity lows reaching -100 mgal. According to Chase and Gilmer (*Earth Planet. Sci. Lett.*, **21**, 70; 1973), however, the shape of the belt may be more significant than the absolute values of gravity. The boundary between the highs and lows is sharply defined by very steep gravity gradients; and when the midcontinent gravity high is mapped by this boundary, it is seen to comprise several long linear segments offset from each other in a way reminiscent of the transform faults along oceanic ridges.

For this and other reasons Chase and Gilmer propose that the rocks produc-

ing the positive gravity anomalies may have resulted from plate tectonic processes which occurred during the Precambrian. That the rocks associated with the anomalies were formed during an episode of continental rifting is not, of course, a new idea in itself. In the Lake Superior region, for example, the positive anomalies lie over uplifted basaltic flows and intrusive gabbros of Keweenawan age, and the gravity lows are found over flanking basins containing younger Keweenawan sediments. The petrology and structure of the volcanics suggest that these rocks were formed during rifting of the Precambrian shield—an emplacement which zircon U-Pb ages show occurred over a short period between 1,140 and 1,120 million years ago. Moreover, evidence from gravity studies, magnetic anomaly data and boreholes indicates that the mid-continent gravity high and rifting are closely associated right through to Kansas.

But what Chase and Gilmer are suggesting is that the acknowledged rifting is the product of the sort of plate tectonic processes known to be occurring today—in other words, that the Keweenawan rift system is the result of the interaction of lithospheric plates during the late Precambrian. According to this view the offsets between the positive anomaly segments would correspond to transform faults and the segments themselves would correspond to oceanic spreading centres. The implication of this would be that the continental regions or blocks on either side of the rift would have acted as rigid plates during the rifting; and this in turn would place constraints on the geometry of the rift. Thus, assuming that these supposedly rigid plates have not been deformed subsequently, the present geometry of the rift should offer a test of the proposed rigidity.

The particular criteria to be applied in such a test are, first, that the supposed transform faults should lie along small circles about the same pole of relative rotation and, second, that the amount of rifting should increase with the sine of the angular distance from this pole. Using five supposed transform faults and the widths of the gravity high measured at eleven points, Chase and Gilmer show that a single pole of rotation may be defined to a remarkable degree of accuracy. The pole itself lies in northern New Mexico at 36°N , 107°W , the total rotation between the block to the north of the gravity high (called the Minnesota plate) and the block to the south (the Wisconsin plate) being 2.6° . At the 95% level the semi-major and semi-minor axes of the pole's oval of confidence are only 3.6° and 1.2° , respectively, and the limits on the rotation are $\pm 0.7^\circ$. The geometrical proper-

ties of the gravity high are thus indeed in close agreement with those to be expected from a plate tectonic model involving interacting rigid plates (apart from in the western Lake Superior region where matters are complicated by the presence of the mafic intrusions of the Duluth complex).

This discovery suggests, in turn, the need for a re-examination of the basic structure of the Keweenawan rift. Previous models of the structure have envisaged a basin 7–15 km deep, filled with dense Keweenawan lavas (supposedly causing the gravity high) and largely underlain by older continental crust. According to this view, the crustal separation would be represented by basaltic feeder dykes beneath the basin, and these would not be clearly defined by the observed gravity. The crustal separation involved in the hidden rifting would thus be difficult, if not impossible, to measure. This is why Chase and Gilmer used the width of the gravity high to represent the degree of rifting, realising that the two were not necessarily equal but assuming that the width of the lava-filled basin would at least be roughly proportional to the lesser width of the rifts hidden below.

But the fact that the width of the gravity high itself is in very close agreement with a plate tectonic geometry implies that this assumption is unnecessary and that the width of the gravity high is actually the width of the hidden rift. Chase and Gilmer thus go on to suggest that the lava basin did not form on a previously existing continental crust which had sunk but instead filled (and fills) the gap between the parted edges of continental plates. In this case it is no longer a matter, as previously thought, of having a relatively minor rift through which lava vented to give a lava basin controlled in width by the previous geology of the region. Instead, the Keweenawan is a major rift which ranges in width from more than 40 km at the southern end in Kansas to more than 85 km in central Lake Superior. A single gravity model is then sufficient to show that such a major rift is as consistent as the previously assumed structure with the observed gravity high.

Finally, from this particular example Chase and Gilmer go on to generalise in terms of an "evolutionary sequence" for continental rifting. They suggest that the initial stages of rifting may resemble the Rhinegraben or Baikal rift, that the next development may be similar to the East African Rift valleys, and that the Keweenawan rift type (the northern Red Sea being another and active example) represents the third stage. The final form would then be the familiar generation of new lithosphere at oceanic ridges as exemplified by today's southern Red Sea and the mid-Atlantic ridge.

Whys and wherefores of cosmic rays

How are cosmic-ray particles produced, and do some of them come from outside our Galaxy? These were the questions implicitly under discussion at a meeting held at the Royal Society on February 20 and 21.

On the second afternoon, T. Gold (Cornell University), V. L. Ginzburg (Lebedev Physical Institute, Moscow) and G. R. Burbidge (University of California, La Jolla) made their own distinctive contributions to the meeting. Gold put forward the case that pulsars could contribute at least electrons, protons and nuclei of mass around that of iron to the cosmic-ray beam. The cosmic radiation requires sources producing about 10^{34} J s^{-1} and the average rate of production of energy by pulsars over a galactic lifetime of 10^{10} yr is reckoned to be 10^{36} J s^{-1} , so energetically the argument holds good. Gold suggested that the early and as yet undetected phases of pulsar evolution might well be instrumental in the formation of other parts of the cosmic-ray flux. He pointed out that there is already evidence from the distribution of pulsar periods, both by number and galactic latitude, that there is more than one way in which a pulsar can be created. The number distribution shows a marked bifurcation at pulsar periods of about 1 s, and Gold said that the probability of such a 'out' in a single modal distribution is about 7%. The fact that pulsars with periods greater than 1 s are much less concentrated in the galactic plane than the others is added evidence of distinct forms of pulsar evolution.

A. W. Wolfendale (University of Durham) said earlier in the meeting that there is evidence of an upward bump in the differential energy spectrum of primary cosmic rays at energies of about 10^{15} eV , and this fitted nicely with Gold's contention that the energy spectrum of cosmic rays from pulsars should be fairly flat, with a cutoff at about that energy.

Ginzburg, whose paper was read in his absence, seems as convinced as he ever was that cosmic rays from outside the Galaxy are unlikely to contribute to the flux inside. He pointed out that if the intergalactic gas density were at the high end of the accepted range, 10^{-7} to $10^{-5} \text{ atoms cm}^{-3}$, this would rule out some extragalactic models straight away. On the experimental side, he described several experiments which could have a crucial bearing on the problem. First, the measurement of the abundance of ^{10}Be (half life $1.6 \times 10^6 \text{ yr}$) would help differentiate between different galactic models. A complete lack of ^{10}Be —indicating that it had all decayed—would

confirm that the halo of the Galaxy is involved in cosmic-ray propagation. Complete survival, on the other hand, would not necessarily rule out models involving the galactic disk as well as the halo. He described the measurement of the isotopic composition of cosmic rays as "on the horizon". Other experiments put forward by Ginzburg included measuring the energy spectrum of relativistic positrons, essentially to find a 'characteristic time' for cosmic rays in the Galaxy and monitoring the γ -ray flux from the galactic centre and from the Magellanic Clouds. The latter would require γ -ray detectors some 300 times more sensitive than those at present available but would place an upper limit on the input of cosmic rays from outside the Galaxy.

Following Ginzburg's advocacy of galactic models, Burbidge put forward the case for thinking that some, though not all, cosmic rays found in the Galaxy

are produced outside its confines. What of the energy implications? Burbidge recalled that filling the whole Universe with cosmic rays at the density found near the Earth ($10^{-5} \text{ J cm}^{-3}$) would require every galaxy to produce 10^{56} J in a lifetime of 10^{10} yr —1% of its rest mass, for some people a worryingly high figure. If, however, calculations are based on the assumption that only galactic clusters and superclusters (1% of the volume of the Universe) are filled with cosmic rays then only 10^{-4} of the rest mass is involved.

Arguing against the objection that extragalactic cosmic rays could not get into the Galaxy, Burbidge said that particles do diffuse into the outer parts of radio galaxies such as Cen A, so that it is difficult to see how particles will fail to get to the Earth. Burbidge appealed to "nature and more theoretical studies", which have up to now tended to be confined to galactic models.

Earthquake history

SEISMOLOGISTS and others interested in earthquake phenomena will probably already know of the two volume *Earthquake History of the United States*, originally published in 1928 and since updated several times. The latest revision brings the data together in one volume, published by the US Department of Commerce (National Oceanic and Atmospheric Administration, 1973).

The data now include earthquakes from the mid-seventeenth century (for the eastern states at least) up to 1970; as well as the catalogue of dates, intensities and places, earthquakes are given short 'eye witness' descriptions as their intensity and destructiveness justify, and maps indicate the 'seismic risk' in different parts of the contiguous United States. Anybody interested in older events (outside of the United States, of course) is referred to Milne's *A Catalogue of Destructive Earthquakes AD7 to AD 1899* (Br. Ass. Advmt. Sci., 649; 1911).



Part of 130 acres of land devastated by 1964 Good Friday shock in Turnagain Heights area of Anchorage, Alaska.

Reality of bubble nuclei

from our Nuclear Theory Correspondent

MANY studies of nuclei by electron and nucleon scattering have shown that some are spherical whereas others are more or less strongly deformed into spheroidal or ellipsoidal shapes. Studies of their excited states show that the deformed nuclei have characteristic rotational spectra but that the spherical nuclei can often be excited into a series of vibrational modes.

Wheeler pointed out long ago that these are not the only conceivable nuclear shapes. One can imagine not only wobbly drops of various shapes but more exotic configurations like bubbles and toroids. Is it possible, for example, to form a bubble of nuclear matter and if so would it be stable?

It is plainly possible to imagine a nucleus from which all the nucleons in the lowest *1s* state have been suddenly removed by a direct interaction, leaving the other nucleons undisturbed. This would be a highly excited nucleus but it might live long enough to qualify as a bubble nucleus. It is also conceivable that bubble nuclei are sometimes formed in the turbulent nuclear motions resulting from very energetic collisions of heavy ions. These bubble nuclei are likely to be highly excited but it is also possible that bubble configurations are found at low excitations or even in the ground state.

Whereas heavy nuclei can usefully be thought of for some purposes as a drop of fluid of almost uniform density, light nuclei have density distributions that can be interpreted as α -like clusters in regular array. Thus ^{12}C has a density distribution similar to that of three α s at the vertices of an equilateral triangle and ^{16}O as four at the vertices of a tetrahedron. Such nuclei may have a low central density and may be classed as bubble nuclei.

Calculations of the stability of bubble nuclei were made by Siemens and Bethe (*Phys. Rev. Lett.*, **18**, 704; 1967) using the semi-empirical mass formula and they found that some large spherical bubble nuclei may be stable against symmetry-preserving breathing expansions and contractions, but they did not investigate their stability against other distortions.

The stability of bubble-shaped nuclei against symmetry-preserving breathing deformations has also been investigated by Krishnan and Pu (*Phys. Lett.*, **47B**, 225; 1973) using the energy density formalism. They find that bubble nuclei are stable only for very large mass numbers ($A \approx 700$), far beyond the region known at present. Lighter nuclei do not have stable bubbles.

A series of calculations using the liquid

drop model shows that bubble nuclei are unstable (Wong, *Ann. Phys.*, **77**, 279; 1973) and this confirms other calculations that do not take the shell structure of nuclei into account. If, however, the shell structure is included, some bubble nuclei with a low density of single-particle states near the Fermi surface are found to be stable. These calculations are made by assuming that the nuclear potential follows the density distribution; the energies of the single-particle states can then be found by standard procedures. The reduced potential near the centre of a bubble nucleus raises the energies of those single-particle states whose wave functions overlap with this region, and these are states of low angular momentum. Thus in a bubble configuration the energies of states of low angular momentum are raised relative to those of states with high angular momentum and this gives a new series of magic nucleon numbers for pronounced bubble configurations.

In this way Wong (*Phys. Lett.* **41B**, 451; 1972) found the new magic numbers 18, 34, 50, 70, 80, 104, 120... at various deformations. Use of the Strutinsky method to evaluate the shell corrections then indicates ^{36}Ar , ^{84}Se , ^{138}Ce and ^{200}Hg as possible bubble nuclei in their ground states.

More detailed calculations may be made with the Hartree-Fock theory using a realistic effective nucleon interaction and some results of Curry and Sprung (*Nucl. Phys.*, **A216**, 125; 1973) for ^{36}Ar are shown in Fig. 1. Also shown is the corresponding distribution for ^{40}Ca less that of an α particle. Allowing for

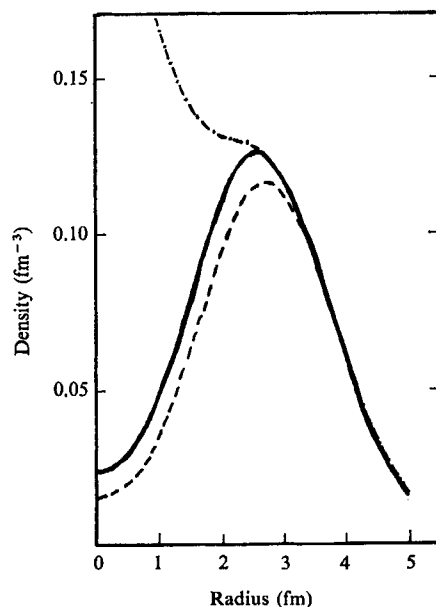


Fig. 1 Density distribution of ^{36}Ar (—) compared with that of ^{40}Ca (---) and the density difference of ^{40}Ca and an α particle (— · —) obtained by Hartree-Fock calculations. The central bubble is well marked, and is identified as an α hole (Curry and Sprung).

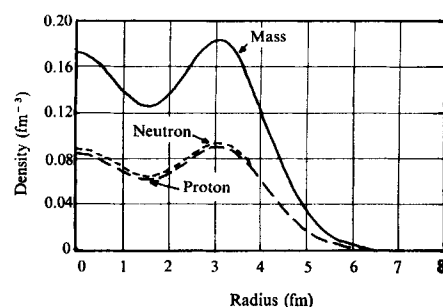


Fig. 2 Density distribution of ^{68}Se showing the region of minimum density centred on a spherical shell of radius about 1.5 fm (Davies, Krieger and Wong).

a slight inward relaxation due to self-consistency effects the two distributions correspond very well, suggesting that ^{36}Ar has a bubble configuration corresponding to ^{40}Ca with an α particle removed from its centre. These nucleons in the α particle are not, however, in *s* states; removal of *s*-state nucleons would give ^{36}Ar in a highly excited state and a density distribution quite different from that shown.

Further calculations for a series of possible bubble nuclei have been made by Davies, Krieger and Wong (*Nucl. Phys.*, **A216**, 250; 1973) and they confirm the bubble nature of a low energy configuration of ^{36}Ar and in addition find marked bubble effects in ^{200}Hg and ^{116}Ce and ^{138}Ce . A different type of bubble is found in ^{68}Se ; the region of lower density is centred on a spherical shell of radius about 1.5 fm instead of the origin (see Fig. 2).

Detailed examination of the results of these calculations shows that in these nuclei the bubble configurations are preferred because they reduce the density of single-particle states in the region of the Fermi surface. The expected raising of states of low angular momentum relative to those of high angular momentum is indeed found in some bubble nuclei. With the same model, the strength of the spin-orbit potential of the Thomas form (proportional to the radial derivative of the density distribution) depends on the relative amplitudes for finding the nucleon at the surface or near the centre of the nucleus. This implies that for bubble nuclei the spin-orbit doublets may be inverted and the likelihood of this is greater for smaller values of orbital angular momentum. This effect is also observed in some nuclei.

These investigations show that the usual picture of nuclei having an almost uniform central density is sometimes far from the truth. There are several nuclei that show pronounced minima or bubbles in their centres and others have even more complicated departures from uniform density.

Evidence for a ~ 4.5 aeon age of plagioclase clasts in a lunar highland breccia

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Argon from neutron-irradiated mineral separates and whole rock samples of a metamorphosed breccia (65015) from Apollo 16 has been analysed with a large number of gas extraction steps in order to obtain a high resolution in the apparent ages and to identify the gas released from different sources. The results on plagioclase show a ^{40}Ar - ^{39}Ar plateau age of 3.98×10^9 yr, which we attribute to the time of metamorphism, and an age of $\sim 4.5 \times 10^9$ yr in the high temperature fraction. Correlation of the release pattern with ^{37}Ar instead of ^{39}Ar permits the association of the $\sim 4.5 \times 10^9$ yr age with relict plagioclasts which were demonstrated in previous petrographic and Rb-Sr studies as being unequilibrated. This result suggests that it is possible to identify lithic components which represent the early lunar crust.

than found in another plagioclase separate, C, which is also closer to the internal isochron. Plag C is itself off the isochron by several times the possible errors, which originally led to the search for the effects in B and subsequently in the more extreme examples of non-equilibration as found in the macroscopically identifiable clasts in the uncrushed rock.

Because of complexities in both the technique (compare ref. 6) and in the breccia itself, Ar analyses were made on a whole sample and several mineral separates of 65015 using a large number of incremental heating steps in order to provide a high resolution in variations of $^{40}\text{Ar}/^{39}\text{Ar}$. It was anticipated that such high resolution measurements, together with other isotopic and petrographic observations, would allow an isolation of the appropriate parts of the 'age spectra' associated with relict retentive phases. To obtain a sharper distinction between the Ar released at low and high temperatures, presumably reflecting the outgassing from lattice sites with different retentivities, we carried out 30–50 degassing steps between $\sim 300^\circ\text{C}$ and $1,500^\circ\text{C}$ on each sample, some of which were repeated extractions at the same temperature. All samples were part of the same neutron irradiation, which minimised comparative errors. The amounts of ^{40}Ar from the radioactive decay of ^{40}K and the amounts of ^{39}Ar produced by neutron capture on ^{39}K were determined after appropriate corrections. (Ar data were corrected sequentially for: spectrometer discrimination, decay of ^{37}Ar ($\lambda_{37} = 0.01975\text{ d}^{-1}$) and ^{39}Ar ($\lambda_{39} = 7.2 \times 10^{-6}\text{ d}^{-1}$) before analysis; Ar interferences from neutrons on Ca and K; normalisation to standard neutron fluence; subtraction of procedural blank with assumed air composition in units of $10^{-9}\text{ cm}^3\text{ STP }^{40}\text{Ar}$ of $(1.5 \pm 0.5) - (2.0 \pm 0.7)$ at $<1250^\circ\text{C}$, (3 ± 1.2) at 1300°C , (7 ± 3) at $1,400^\circ\text{C}$, (17 ± 8) at $1,500^\circ\text{C}$. We used: $(^{36}\text{Ar}/^{37}\text{Ar})_{\text{Ca}} = 0.000305$, $(^{39}\text{Ar}/^{37}\text{Ar})_{\text{Ca}} = 0.000732$, $(^{38}\text{Ar}/^{39}\text{Ar})_{\text{K}} = 0.01$, $(^{40}\text{Ar}/^{39}\text{Ar})_{\text{K}} = 0.01$.) The apparent ages were calculated from the $^{40}\text{Ar}/^{39}\text{Ar}$ ratios for each temperature release and are shown in Fig. 1A for the whole rock and two mineral separates. (Conversion factors for LAV3 irradiation: $C_{\infty}(\text{K}) = 8.02 \times 10^{-4}\text{ cm}^3\text{STP }^{39}\text{Ar/gK}$, $\text{K/Ca} = (0.54 \pm 0.02)$ $^{39}\text{Ar}/^{37}\text{Ar}$. Constants in age calculations: $\lambda_p = 4.27 \times 10^{-10}\text{ yr}^{-1}$, $\lambda_o = 0.585 \times 10^{-10}\text{ yr}^{-1}$, $^{40}\text{K/K} = 1.19 \times 10^{-4}$.) The age scale is enlarged to make the fine structure visible.

The apparent ages of all samples, including two which are not illustrated, increase very rapidly to a local maximum at $\sim 600^\circ\text{C}$, followed by a decrease of $(0.05\text{--}0.1) \times 10^9$ yr. The apparent ages of the plagioclase and whole rock recover above about 840°C , whereas those of the pyroxene remain almost constant up to 900°C . A normal experiment with less resolution would not show any of this fine structure, but would give very well defined plateaux of $(3.90\text{--}4.05) \times 10^9$ yr for all samples between $500\text{--}900^\circ\text{C}$. Above 900°C , the ages of both pyroxene and whole rock decrease by $\sim 0.8 \times 10^9$ yr and $\sim 0.3 \times 10^9$ yr, respectively, whereas plagioclase exhibits a rapid increase in apparent age. The highest age of 4.5×10^9 yr is approached in a regular sequence of precise data which, except for the very last point, are not affected by blank corrections (Fig. 1B). Because the total rock is a polymineralline aggregate, its complex behaviour is not un-

THIS investigation attempts to decipher the premetamorphic age of the protolith of a recrystallised breccia from Apollo 16 using the ^{40}Ar - ^{39}Ar technique. Petrographic studies have revealed in some lunar breccias, clasts which have not been brought into chemical equilibrium with the recrystallized groundmass¹. Detailed Rb-Sr studies have shown that some plagioclase clasts were not isotopically equilibrated with the matrix and consequently show isotopic evidence of a pre-metamorphic history^{2,3}. The study of such unequilibrated rocks is of major importance in penetrating the time barrier at $\sim 4.0 \times 10^9$ yr, reflecting the terminal lunar cataclysm³⁻⁶. Such results are directly applicable to the characterization of the early lunar crust and the time scale for its evolution.

Rock 65015 was known to contain Sr isotopic relicts in plagioclase mineral separates⁴ and was therefore selected for a comprehensive study with the ^{40}Ar - ^{39}Ar technique⁷. An optimal experiment would be an analysis of macroscopically recognisable plagioclase clasts which were known to be almost totally unequilibrated. Such a sample of 0.5 mg weight has provided the nearest 65015 data point to the primitive Sr isotopic composition of BABI (see Fig. 1 of Tera *et al.*⁴). A similar ^{40}Ar - ^{39}Ar analysis unfortunately requires much more material than is currently available. As a compromise, a mineral separate was chosen which was relatively large in size (47 mg) but which was still known to be distinctly off the internal Rb/Sr isochron by a small amount. This sample (plag B) may be seen in fig. 4 of Papanastassiou and Wasserburg² and fig. 1 of Tera, *et al.*⁴ Although it may superficially appear that this sample lies on the internal isochron, it must be recognised that the analytical errors are the size of the central dots. Further, the five different plagioclase separates lie precisely on a straight line which is quite different from the internal isochron, and the position of plag B along this line indicates that about 14% of plag B is composed of unequilibrated clasts as distinct from groundmass plagioclase. This was supported by inspection of the mineral separate B, which showed a higher ratio of large ($\sim 100\text{ }\mu\text{m}$) plagioclase cleavages to finer grained groundmass plagioclase

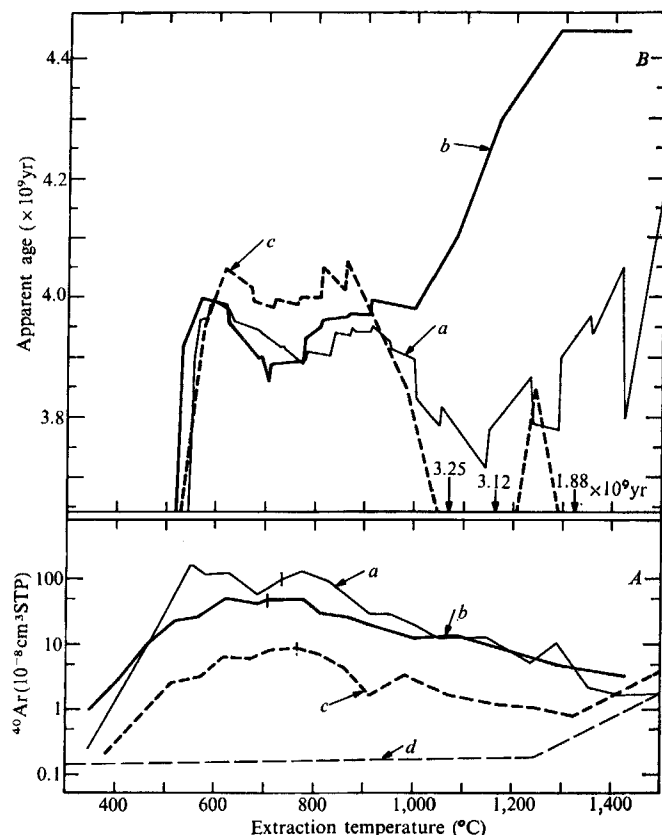


FIG. 1 Differential ^{40}Ar release (A) and apparent ages (B) against extraction temperature for: a, total rock sample (64 mg); b, plagioclase B (47 mg); c, pyroxene (57 mg) separates of Apollo 16 breccia 65015; d, blank. A, The amounts of ^{40}Ar released in multiple extractions at the same temperature have been added. Vertical bars mark the temperature where $\sim 50\%$ of the total ^{40}Ar has been degassed and illustrate that the low temperature release of all samples is dominated by a high K phase. In the plagioclase curve at $\sim 1,000^\circ\text{C}$ there is a hint of a structure possibly due to the release from a high temperature 'phase'. The average blank amounts, determined from 35 procedural blank runs with maximum variations of 50% from the given curve, affect only the last extraction of each sample, but clearly not the $1,290^\circ\text{C}$ plagioclase datum. B, To separate visually the apparent age points from multiple extraction steps at constant temperature T , the age of the n th step is plotted at $(T + 5n)^\circ\text{C}$ on an expanded age scale covering the significant range. The low temperature apparent age patterns are characterised by a similar behaviour for all samples, which we attribute again to an ubiquitous high K and relatively unretentive mineral phase. Above $\sim 900^\circ\text{C}$ pyroxene shows its characteristic decrease, which is not understood. Plagioclase, on the other hand, after a well documented plateau ($865\text{--}1,000^\circ\text{C}$) increases rapidly to 4.46×10^9 yr at $1,290^\circ\text{C}$. The whole rock age pattern is a complex synthesis of all phases.

expected at this resolution. The high temperature decrease in the age of the whole rock sample can be attributed to the inclusion of pyroxene, for which the decrease at high temperatures is here again established as characteristic (see ref. 8), although it is not yet understood. The maximum ages at 600°C differ by up to 0.05×10^9 yr, but the similarity in the low temperature behaviour of all samples suggests a source for this characteristic pattern in a ubiquitous accessory phase which is not readily removed by mineral separation techniques on such fine grained material. Albee *et al.*¹ have noted that 90% of the potassium in 65015 resides in non-stoichiometric, irregularly dispersed, very fine patches, which appear to be devitrified glass (quintessence). This phase contributes a large fraction of the total ^{40}Ar released at low temperatures (Fig. 1B), as demonstrated by the fact that at low temperatures K/Ca is 10–100 times larger than at high temperatures (Fig. 2C).

In summary, we conclude that by usual standards, the $^{40}\text{Ar}/^{39}\text{Ar}$ ratios in the region between 550 to 900°C define reasonable plateaux corresponding to ages between 3.90×10^9 yr and 4.05×10^9 yr. The complex age spectra, however, are apparently the result of a blend of a common K-rich phase with the major minerals plagioclase and pyroxene. It is evident from these refined age patterns that a variety of processes must be taking place during the differential thermal analyses. The causes for the complex age spectra are not well established. A reasonable mechanism to explain the characteristic age peak at $\sim 600^\circ\text{C}$ is redistribution of ^{39}Ar because of recoil from fine grained quintessence. Typically, the low initial ages would be interpreted as a result of ^{40}Ar loss on the lunar surface. Because of the low temperature fine structure, and the possibility of ^{39}Ar redistribution, the exact extent of any ^{40}Ar loss on the lunar surface is uncertain.

We will now focus on the plagioclase results and the study of Ar released from plagioclase in the dominating presence of Ar released from other phases. It is our purpose to identify the plagioclase ages and separate them from the ages of high K impurities. Traditionally, the apparent ^{40}Ar – ^{39}Ar ages are plotted against the cumulative fractional ^{39}Ar release (Fig. 2A). In polymineralic systems containing minerals with very different K concentrations, the age pattern in this representation may be dominated by rare high K phases, which have a preponderance of total K in the sample.

Evidence for this is seen both in the fact that the total K in plagioclase separate B inferred from total ^{39}Ar is 1,475 p.p.m., in contrast with ~ 500 p.p.m. for plagioclase in 65015 determined by electron microprobe¹, and in the fact that the dominant ^{39}Ar and ^{40}Ar release occurs at low temperatures (Figs 1A and 2A). As plagioclase B is $\sim 99\%$ pure, this would require that the additional phase which releases argon at low temperatures can only amount to about one percent of the sample weight but must have a very high K content. An admixture of only 1.5% of quintessence with 6.4% K (ref. 1), can account for two-thirds of the K in plagioclase B.

In contrast to the potassium, Ca in the plagioclase is $\sim 13\%$ as compared to only $\sim 2\%$ in quintessence¹. Therefore ^{37}Ar release from our sample is related almost entirely to the release from plagioclase and not the K-rich, low Ca impurities. A true plagioclase plateau should be associated with the ^{37}Ar released. Figure 2B shows the data in this representation. There is a distinct low temperature peak in the high K/Ca regime (fig. 2C) and a reasonably well defined plateau over the temperature region $865\text{--}1,000^\circ\text{C}$, containing 35% of the ^{37}Ar and corresponding to an almost constant K/Ca ratio of 0.005, which is appropriate to plagioclase. It now becomes evident that the $\sim 600^\circ\text{C}$ peak is a disturbance. Since it covers approximately the first 50% of the ^{39}Ar release (Fig. 2A), it would normally be interpreted as an intrinsic part of the plateau, but it is identified in the ^{37}Ar plot as a minor contribution from a high K phase in the age spectrum of plagioclase. In the high temperature regime, the sharp increase of the apparent age to 4.5×10^9 yr is

TABLE 1 Argon ages in breccia 65015 (inferred from plagioclase mineral separate B)

	Gas retention age ($\times 10^9$ yr)*	Exposure age (m.y.)†
865–1,000° C		
'equilibrated plagioclase'	3.98 ± 0.01	466 ± 1
1,290° C		
'unequilibrated plagioclase clasts'	4.46 ± 0.05	≥ 505

* When comparing these ages with those from other laboratories or other irradiations, an additional error of $\pm 0.04 \times 10^9$ yr from the uncertainty in C_{39} (K) has to be included.

† Calculated with production rate $P_{38} = 1.4 \times 10^{-8}$ cm³ STP $^{38}\text{Ar}/\text{g Ca my}$ and excluding an error of ± 17 m.y. from C_{37} (Ca).

more obvious and is correlated with the last 15% of the ^{37}Ar release, as compared with the last 5% of the ^{39}Ar release. It should be noted that these observations depend critically

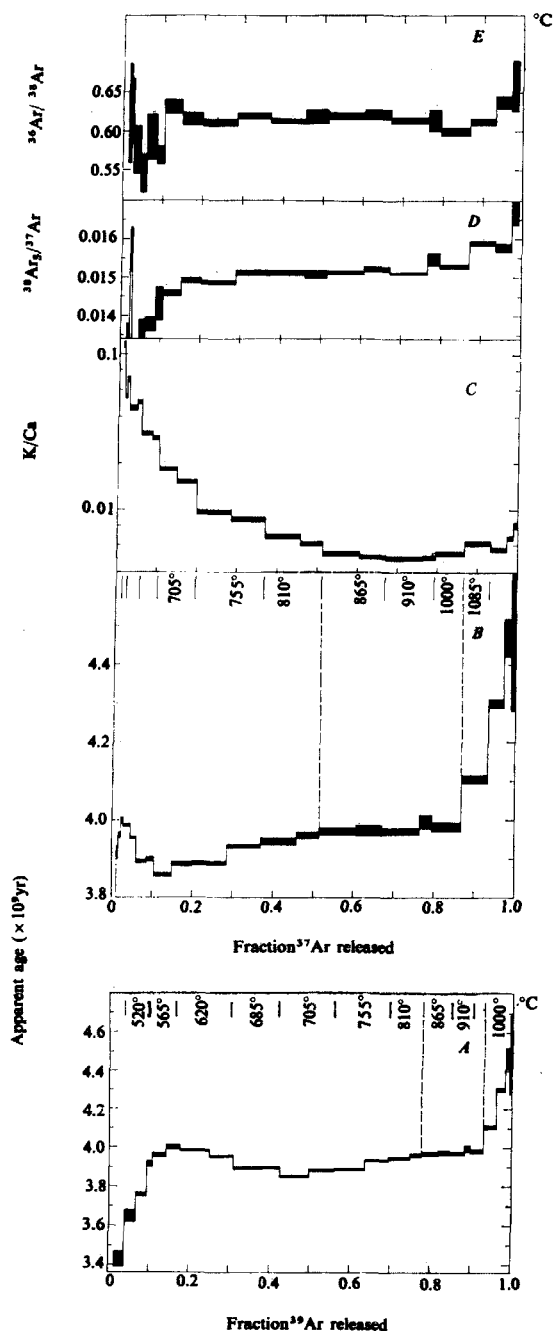


FIG. 2 Isotopic composition of Ar from a plagioclase separate of Apollo 16 breccia 65015, against (A) fractional release of ^{39}Ar , and (B-E) fractional release of ^{37}Ar . The graph of apparent age against ^{39}Ar (A) is dominated by Ar released from K-rich, Ca-poor accessory phases. Graphs against ^{37}Ar emphasise the composition of Ar from Ca-rich plagioclase and reveal (between vertical dashed lines) a well-defined intermediate plateau at 3.98×10^9 yr (B). This is associated with the appropriate K/Ca for plagioclase (C), a constant $^{36}\text{Ar}/^{38}\text{Ar}$ (D) at a value appropriate for spallogenic Ar, and a well defined plateau in $^{38}\text{Ar}_s/^{37}\text{Ar}$ (E) indicating a cosmic-ray exposure age of 466 m.y., all ascribed to plagioclase degassed or recrystallised by metamorphism at 3.98×10^9 yr. The last 10% of ^{37}Ar release shows increases in apparent age associated with increases in K/Ca and $^{38}\text{Ar}_s/^{37}\text{Ar}$, but constant $^{36}\text{Ar}/^{38}\text{Ar}$. We attribute this to unequilibrated and incompletely degassed plagioclase clasts from the protolith of 65015 previously recognised by petrographic and Rb/Sr studies, and infer for them a formation age of $\sim 4.5 \times 10^9$ yr, and exposure to cosmic rays before 3.98×10^9 yr.

on high resolution data. If there were only ten equal steps, then the last four releases would have given an apparent age of 4.2×10^9 yr.

It is most plausible to associate the plateau of 3.98×10^9 yr on the ^{37}Ar plot with plagioclase which has completely lost ^{40}Ar in a metamorphism at that time, and to further associate the age peak at $\sim 600^\circ\text{C}$ with the crystallisation of quintessence at approximately the same time. The age of 4.5×10^9 yr obtained in the highest temperature release from the plagioclase separate is one of the most interesting and possibly most important results of the present work. It is plausible to correlate this age with the plagioclase clasts which have not been thoroughly equilibrated and outgassed during this metamorphism. It is conceivable, however, that the high age values are artefacts. As a test of this, the $^{36}\text{Ar}/^{38}\text{Ar}$ and $^{38}\text{Ar}_s/^{37}\text{Ar}$ ratios can be inspected for irregularities (see Figs. 2D and E). Again the ^{37}Ar plot is the most convenient representation, since Ca is the predominant target nucleus for the production of spallogenic Ar by cosmic rays. It can be seen that the $^{36}\text{Ar}/^{38}\text{Ar}$ ratio is remarkably constant at 0.62, in good agreement with typical spallation values on Ca and Fe (ref. 9). There is a hint of slight fluctuations ($\sim 6\%$) in the last 10% of the ^{37}Ar release. The $^{38}\text{Ar}_s/^{37}\text{Ar}$ shows a broad plateau after the first 10% and up to the last 15% of ^{37}Ar release. The pattern of $^{38}\text{Ar}_s/^{37}\text{Ar}$ characterises a well defined cosmic-ray irradiation age, with spallation $^{38}\text{Ar}_s$ exactly correlated with the Ca target, as measured by ^{37}Ar . The last $\sim 15\%$ of the ^{37}Ar release shows a small but distinct ($\sim 10\%$) increase in $^{38}\text{Ar}_s/^{37}\text{Ar}$ correlating with the high $^{40}\text{Ar}/^{39}\text{Ar}$ part of the age spectrum and also some variations in K/Ca. The most obvious interpretation is to consider this as reflecting a pre-irradiation of the older plagioclase clasts before the formation of the recrystallised breccia, in which case the clasts must have resided for some time in the upper few metres of the lunar surface before the last major collision causing the metamorphism. The increase in $^{38}\text{Ar}_s/^{37}\text{Ar}$ cannot be due to a marked change in chemistry (for example, Fe or Ti enrichment¹). A production of ^{38}Ar by neutron reactions on ^{37}Cl is highly improbable since $^{36}\text{Ar}/^{38}\text{Ar}$ is constant. If the $^{38}\text{Ar}_s/^{37}\text{Ar}$ increase is an artefact, it is a factor of two smaller than that manifested in the $^{40}\text{Ar}/^{39}\text{Ar}$ ratio, and a factor of $\sim 10^2$ smaller in the number of ^{40}Ar atoms as compared with the ^{38}Ar . Thus we do not consider the change at high temperatures in the ratios of the spallogenic isotopes to be indicative of artefacts in $^{40}\text{Ar}/^{39}\text{Ar}$ ratios. The average K/Ca is 20% higher in the plagioclase which apparently retains Ar from dates before 3.98×10^9 yr. Observations on the cores of plagioclase clasts show that they are usually slightly lower in K than the rims and matrix plagioclase, although some do have relatively higher K contents.

The correlation of gas release from plagioclase with the ^{37}Ar release enables us to estimate that $\sim 15\%$ of plagioclase B can be related to high ages, which is in striking agreement with $\sim 14\%$ inferred from Rb/Sr studies of the same separate. This provides another very important and independent argument for the association of the $\sim 4.5 \times 10^9$ yr ages with unequilibrated plagioclase clasts. The argument is considerably strengthened by the observation of a similar $^{40}\text{Ar}/^{39}\text{Ar}$ increase to $\sim 4.1 \times 10^9$ yr in approximately the last 5% of the ^{37}Ar release from plagioclase C, which Rb/Sr studies indicate contains only $\sim 8\%$ unequilibrated clasts.

Thus, the analysis of the high resolution differential thermal release patterns of all Ar isotopes from neutron irradiated samples of the lunar rock 65015 indicates that this breccia was metamorphosed and recrystallised 3.98×10^9 yr ago, at about the time of the terminal lunar cataclysm. Part of the plagioclase shows a distinctive high temperature age maximum of 4.46×10^9 yr which is interpreted as being due to incomplete outgassing of old plagioclase clasts

during the metamorphic event. These results are in support of the reports by other workers^{8,10} on evidence for more ancient ages in some lunar samples. In the instance of 65015, the ages gain credence from corroborating petrographic and Sr isotopic studies, but the firm assignment of a precise time to some part of a ⁴⁰Ar-³⁹Ar release pattern requires a complete understanding of the nature of ⁴⁰Ar/³⁹Ar variations. The plagioclase clasts are tentatively considered to have original ages of formation of at least 4.46×10^9 yr and therefore provide evidence for the existence of some early Lunar crust with plagioclase of this composition formed 4.5×10^9 yr ago. There is no evidence in the data for intermediate metamorphic events. The data are consistent with a simple history of formation of some constituents of the breccia before $\sim 4.5 \times 10^9$ yr and subsequent formation of the breccia followed by severe metamorphism 3.98×10^9 ago. This is in agreement with a two stage evolution model for the lunar crust with these identical age limits established by Pb/U, Th systematics for an extensive suite of samples which includes 65015.

The high ⁴⁰Ar-³⁹Ar age of part of the plagioclase is accompanied by a slightly older exposure age relative to the rest of the breccia, which is indicative of a pre-irradiation. Generalising the 65015 results, we propose that the protolith of many impact breccias includes in part material which resided for some time in the outer few meters of the lunar surface before the metamorphism which resulted from major impacts $\sim 4.0 \times 10^9$ yr ago.

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Evidence for continental crust beneath the Faeroe Islands

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Recent crustal seismic refraction experiments in the North Atlantic indicate that the Faeroe Islands are underlain by continental crust. This suggests that the whole Rockall-Faeroe Plateau may be a microcontinent which formed during the early evolution of the North Atlantic.

THE Faeroe Islands lie at the north-eastern end of a region of shallow banks in the North Atlantic known as the Rockall-Faeroe Plateau (Fig. 1), which is separated from the United Kingdom continental shelf by the Rockall Trough and Shetland-Faeroe Channel. It has recently been demonstrated that Rockall Plateau at the south-western end of this region is underlain by ancient continental crust¹⁻³. The Faeroe Islands also lie at the south-eastern end of another shallow feature known as the Iceland-Faeroe Ridge, but this is fundamentally different from Rockall Plateau in that it is underlain by anomalous Icelandic-type oceanic crust formed by differentiation from the mantle over the last 60 m.y., during the evolution of the North Atlantic by seafloor spreading^{4,5}. The

Faeroe Islands themselves are formed by a thick pile of nearly flat-lying lavas of early Tertiary age whose base is not seen⁶; the exposed lavas are radiometrically dated⁷ at 50 to 60 m.y. which makes them contemporaneous with the early Tertiary volcanic activity of north Britain and Greenland. The nature of the crust beneath the Faeroes is thus problematical—is it continental or anomalous oceanic? The answer is crucial to our understanding of the Tertiary development of the north-eastern North Atlantic. We give new evidence from a crustal seismic refraction experiment which indicates that the lavas of the Faeroe Islands are underlain by continental crust about 30 km thick.

Before the present work, two items of evidence indicated that the Faeroe Islands may be continental. First, Bott and Watts⁸ showed, in making a pre-Tertiary continental reconstruction of the north-eastern North Atlantic, that if Rockall Plateau is regarded as continental then the Faeroe Block must also be continental to give an acceptable fit with Greenland. Second, a steep Bouguer anomaly gradient occurs between the Iceland-Faeroe Ridge and the Faeroe Block, indicating that the mean crustal density is significantly less beneath the block than the ridge and suggesting funda-

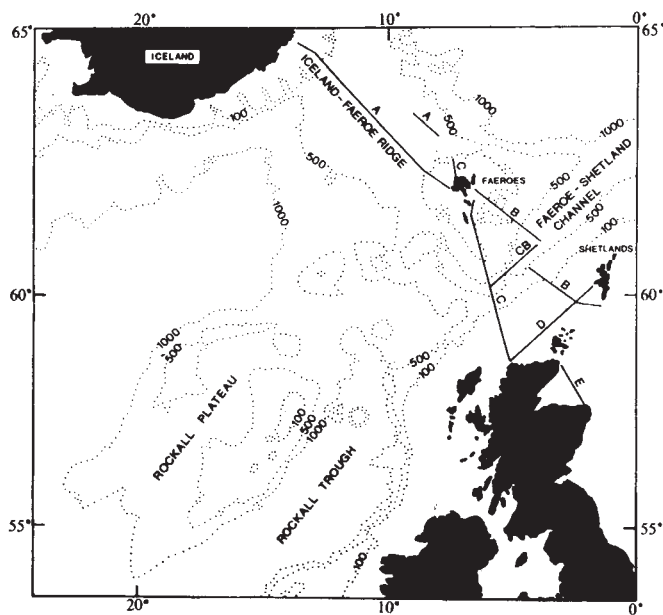


FIG. 1 Map of the region between Scotland and Iceland, showing the position of the Faeroe Islands in relation to Rockall Plateau and the Iceland-Faeroe Ridge, and the lines of shots fired during the North Atlantic Seismic Project. The following bathymetric contours are shown: 100, 500 and 1,000 fathoms.

mentally different types of crust beneath the two adjacent regions^{4,5}. Conversely, an early seismic refraction experiment made by Pálmason⁹ gave a 6.4 km s^{-1} basement beneath 2.5–4.5 km of lavas, indicating a structure similar to that beneath Iceland¹⁰. Another seismic experiment made by Casten¹¹, however, gave an unreversed basement velocity of 5.9 km s^{-1} which would be in accord with normal continental crust.

During a major seismic refraction project between Scotland and Iceland in 1972, geophex shots of 300 to 2,000 pound were fired along the lines A to E shown in Fig. 1. Recording stations were situated on Iceland, Faeroe Islands, Shetland and Orkney Islands, and the Scottish mainland. Shots were also recorded at sea by MV *Miranda* and the Russian ship *Michail Lomonosov*. Seven recording stations were placed on the Faeroe Islands, six provided by Aarhus University (in cooperation with Hamburg and Kiel Universities) and one by Durham (Fig. 2). A preliminary interpretation of that

part of the project which concerns the crustal structure of the Faeroe Block is given in this paper, using data received at four of the Faeroe stations, the ship *Miranda* and the United Kingdom Atomic Energy Authority (UKAEA) array station on the Shetland Islands (Fig. 2).

The P_g arrivals from shots on the Faeroe shelf fall into two groups as follows: (1) those from shots on the north Faeroe shelf, and (2) those from shots to the east and south of the Faeroe Islands. These two groups need to be treated separately. The records from shots on the north Faeroe shelf yield apparent velocities as follows: line A: DU4– $6.17 \pm 0.14 \text{ km s}^{-1}$, F1– $5.98 \pm 0.08 \text{ km s}^{-1}$, F2– $6.18 \pm 0.12 \text{ km s}^{-1}$, *Miranda*– $5.98 \pm 0.22 \text{ km s}^{-1}$; line C (north-west): DU4– $5.92 \pm 0.08 \text{ km s}^{-1}$. The observations at station F1 and on *Miranda* roughly reverse line A suggesting a true P_g velocity of 5.95 km s^{-1} between these stations. A time-term analysis¹² on all the north Faeroe shelf data available, including that of Pálmason⁹, yields a value of P_g velocity of $6.18 \pm 0.09 \text{ km s}^{-1}$. We have looked again at Pálmason's earlier results⁹, and we find that if first arrivals only are used then the velocity of the basement beneath his reversed line is 5.9 km s^{-1} . Thus our results suggest that the true P_g velocity in this northern region is within the range 5.9 to 6.2 km s^{-1} . This value is significantly lower than the velocity of 6.4 km s^{-1} for the lower crustal layer beneath Iceland but it is fairly typical of metamorphic basement rocks of the continental crust. The time terms for individual shots and stations in this region are of the order of 0.3 to 0.6 s, which are consistent with a varying thickness of between 2.5 and 5.0 km of lavas of average velocity 4.9 km s^{-1} overlying a basement of velocity 6.1 km s^{-1} . Alternatively a thinner lava sequence may be separated from the basement by pre-Tertiary sediments forming a low velocity layer.

Arrivals of P_g waves from shots to the south and east of the Faeroe Islands yield apparent velocities as follows: line B (shot times corrected for known sediment thicknesses): DU4– $5.28 \pm 0.02 \text{ km s}^{-1}$, F2– $6.00 \pm 0.08 \text{ km s}^{-1}$ from 0 to 50 km and $5.31 \pm 0.04 \text{ km s}^{-1}$ from 50 to 110 km; line C: DU4– $5.52 \pm 0.08 \text{ km s}^{-1}$, F2– $4.97 \pm 0.04 \text{ km s}^{-1}$, F6– $5.38 \pm 0.06 \text{ km s}^{-1}$. A time-term analysis on all the data here yields a refractor velocity of $5.53 \pm 0.08 \text{ km s}^{-1}$. The arrivals from line B recorded at station F2 (Fig. 3) are particularly significant, indicating that the 6 km s^{-1} basement beneath the northern region gives way to a lower velocity basement which occurs to the south-east of a line very approximately passing beneath shot 3 of line B in a NE-SW direction. The 5.3 to 5.5 km s^{-1} basement refractor to the south-east of this line could be interpreted as a folded series of shales, greywackes, slates and so on striking in a north-east to southwest direction. No higher velocity first arrivals attributable to P_g have been observed, implying that the 5.4 km s^{-1} layer must extend to substantial depth.

Shots on lines B and C on the Faeroes shelf beyond a distance of about 110 km give rise to the Moho head wave P_n observed at stations DU4 and F2 on the Faeroe Islands (Fig. 3). This data by itself is not adequate to determine satisfactorily the crustal structure of the Faeroe Block, but fortunately good P_n arrivals from shots on the northern and eastern parts of the Faeroe shelf were observed at the Shetland array station. We have therefore carried out a time-term analysis of P_n arrivals at stations DU4 (=B1), F2 (=C54B) and UKAEA for the following shots: line A 1–6, line B 1–16, line C 54A, 54C and 55–59. The resulting estimate of P_n is $8.24 \pm 0.35 \text{ km s}^{-1}$ and, excepting an anomalous low value of 2.83 s at shot A1, all the time-terms on the Faeroe Block lie between 3.15 and 4.00 s. The mean time-term is 3.60 s which yields a mean crustal thickness of 33 km for an average crustal velocity of 6.1 km s^{-1} , or 40 km for 6.6 km s^{-1} . The mean time term for the stations and shots in the immediate vicinity of the Faeroe Islands (that is, stations DU4 and F2, shots A1–3, C54A, C54C, C55 and C56)

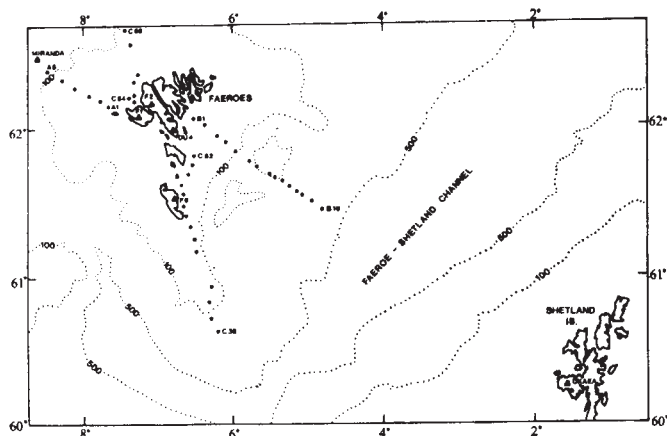


FIG. 2 Map showing the shot positions (●) and seismic recording localities (▲) used in the present analysis. 100 and 500 fathom bathymetric contours are shown. Shots B6, C40, C53 and C57 were misfires and are not shown.

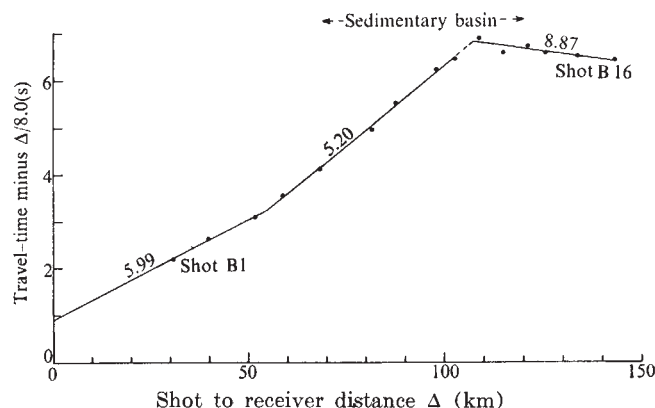


FIG. 3 Reduced time-distance graph for shots B1 to B16 at station F2 on the Faeroe Islands.

is 3.45 s, yielding crustal thickness estimates of 31 km for 6.1 km s^{-1} and 38 km for an average crustal velocity of 6.6 km s^{-1} . If, on the other hand, the true P_n velocity is 8.0 km s^{-1} , then the average time terms are slightly reduced and the resulting crustal thickness estimates are about 5–10% lower than those given above.

Although the velocity of P_n is not particularly well determined, the time terms are determined with some confidence. Depending on assumptions of true P_n value and mean crustal velocity, the crustal thickness beneath the Faeroe Islands is estimated to lie between about 27 and 38 km. There is no evidence for a 6.4 to 6.8 km s^{-1} layer at fairly shallow depth, such as gives rise to clear first arrivals on Iceland and the northern part of the Iceland-Faeroe Ridge, but this does not exclude the possibility that such higher velocities occur in the lower part of the crust.

Our results show that the crust beneath the Faeroe Block differs in four important respects from the anomalous oceanic crust beneath Iceland¹⁰ and the Iceland-Faeroe Ridge⁴: (1) P_n velocities of 5.9 to 6.2 km s^{-1} and less are characteristic of the Faeroes but velocities within this range have not been observed beneath Iceland; (2) a 6.4 to 6.8

km s^{-1} layer (oceanic crustal layer 3?) is present beneath Iceland and the Ridge but has not been observed at a shallow depth beneath the Faeroes; (3) a high sub-Moho P_n velocity of 8.2 km s^{-1} is observed for the Faeroes, in contrast to the anomalously low value of 7.2 km s^{-1} beneath Iceland; (4) the Faeroes' crustal thickness of about 30 km is substantially greater than that beneath Iceland. Our results thus seem to confirm that continental crust underlies the Faeroe Islands and the adjacent shelf regions. This suggests that the Rockall-Faeroe Plateau as a whole may form a single microcontinental fragment which originated as a result of the early stages in the evolution of the north-eastern North Atlantic, with subsequent strong subsidence affecting parts of the region between Rockall Plateau and the Faeroes.

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Direct physical evidence for secondary structure in an isolated fragment of R17 bacteriophage mRNA

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The region of R17 RNA protected from RNase digestion by coat protein assumes a stable, predictable secondary structure. It seems that this structure is recognised by the protein when functioning as a translational repressor.

NUCLEOTIDE sequence analysis of naturally occurring RNA molecules generally reveals sequences which may be arranged into plausible secondary structures. In the case of the RNA bacteriophages, specific structures have been suggested for many segments of the single-stranded genome, including the three ribosome binding sites¹, extracistronic regions at the 5' and 3' termini²⁻⁶, and the phage coat pro-

tein cistron⁷⁻⁹. Recently, Bernardi and Spahr¹⁰ characterised a 59-nucleotide fragment from R17 RNA which is specifically protected from nuclease digestion in the presence of coat protein. Thermodynamic rules for the evaluation of RNA secondary structures¹¹ predict a structure for the fragment consisting of two very stable hairpin helices (see Fig. 1). We have investigated the secondary structure of the isolated fragment by physical techniques and find close agreement between the observed properties of the RNA and those expected based on the proposed secondary structure. Magnetic resonance measurements reported in an accompanying paper give further strong support for secondary structure¹².

Isolation of fragment

To prepare the coat protein-protected RNA fragment in quantities sufficient for physical studies, the original procedure of Bernardi and Spahr¹⁰ was modified slightly (see legend to Fig. 1). The 59-nucleotide fragment and a smaller variant with 29 nucleotides and lacking helix *a* of Fig. 1 were obtained by polyacrylamide gel electrophoresis. The larger fragment used for physical studies was estimated to be 90% homogeneous by both autoradiography and RNA staining. *T*₁ and pancreatic RNase fingerprints showed that the sequence corresponds to that analysed by Bernardi and Spahr¹⁰ except that the end of the coat cistron shows the C → U base change originally observed by Nichols⁸.

Demonstration of secondary structure

The purpose of the physical measurements described here is to observe thermal unfolding of RNA secondary structure; we then draw conclusions about what structure is present from how it unfolds. Application of simple theory to the data allows one to conclude how many hairpin helices are present and their approximate size. Since these numbers are consistent with the structure in Fig. 1, our results provide

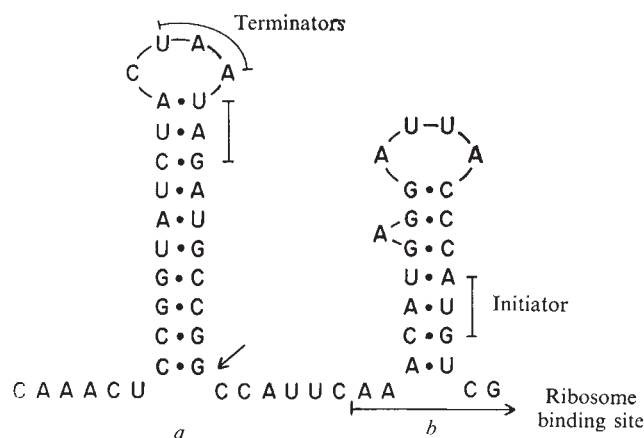


Fig. 1 Sequence and proposed secondary structure of the mRNA fragment from phage R17. The hairpin helix *a* includes the termination codons for the coat protein gene. The hairpin helix *b* includes part of the intercistronic region as well as the initiator codon for the replicase gene. The arrow indicates the site of *T*₁ RNase cleavage which produced the 29 nucleotide fragment. The area protected from pancreatic RNase cleavage by ribosome binding¹ is also indicated. Large quantities of the RNA fragment were prepared as follows. R17 phage were grown and purified²⁰. The complex between R17 RNA and coat protein²¹ was formed at 0° C in 10 ml of TMK buffer¹⁰ (0.1 M Tris-HCl, pH 7.5, 0.01 M Mg acetate and 0.08 M KCl) containing 55 mg of R17 RNA (to which 8×10^7 c.p.m. ³²P-RNA had been added) and 0.8 mg of R17 coat protein. After 10 min, *T*₁ RNase (Sankyo) was added at 0.1 mg ml⁻¹ and incubated for 30 min at 25° C. After a 10-fold dilution with TMK buffer, the preparation was filtered slowly through 24 Millipore filters (HA, 25 mm), rinsed and extracted as described by Bernardi and Spahr¹⁰. Electrophoresis on a 12.5% polyacrylamide gel²² (20 × 20 × 0.1 cm) yielded two bands. Substitution of TMK by buffer I (see Fig. 2), which was used in the physical studies, during the formation and digestion of the complex gave comparable results. Electrophoretic elution of the upper band into dialysis tubing in the presence of 0.02 M Tris acetate, pH 8.0, yielded 270 μg of the isolated fragment. Aliquots of the fragment were digested with *T*₁ or pancreatic RNase and fingerprinted by standard techniques. In the pancreatic map, GGC and GGU were obtained in equimolar amounts; this observation plus the 3:1 ratio of U:C found in UAUCUACUA-AUAG, demonstrated that the sequence in the coat cistron terminator region is that originally established by Nichols⁸.

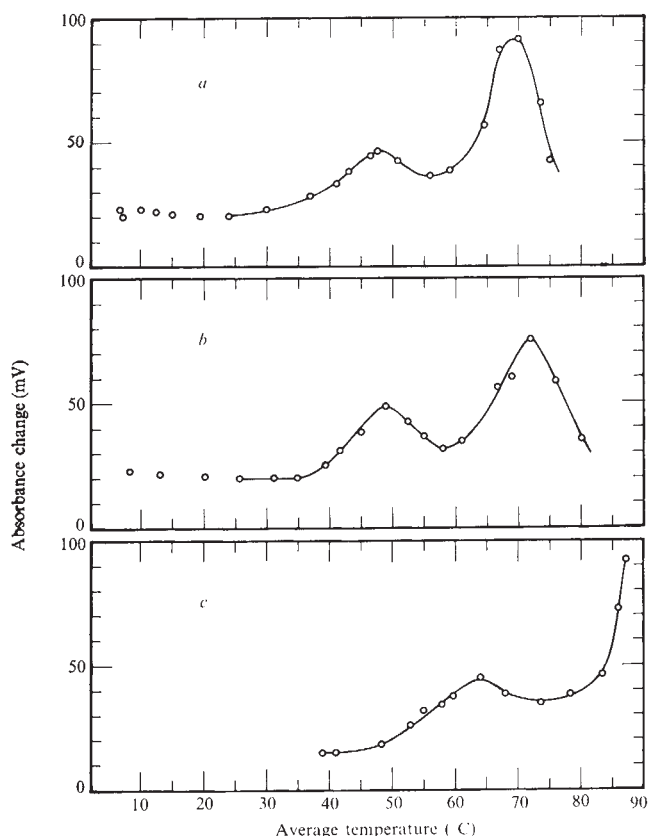


Fig. 2 Differential melting curves for the RNA fragment obtained by the temperature-jump technique. Each point represents the absorbance rise measured following a 4.6° C temperature jump. The total amplitude of the relaxation signal in mV at 266 nm is plotted against the temperature corresponding to the midpoint of the temperature interval. Curves were normalised to an absorbance of 0.45 at 266 nm and 25° C. In the case of curve *a*, data were sufficiently accurate to allow partitioning of the total absorbance change into the slow (helix-coil) and fast (unstacking) components. A plot of the slow amplitude against temperature was then used for the calculation of thermodynamic parameters as reported previously¹³. Peaks in these curves are identified as the melting temperatures, except for curve *c* where the transition could not be followed above 90° C. In this one case, the upper *T*_m was estimated by identifying the temperature at which the ratio of the peak amplitudes was similar to the ratio in curve *a*. *a*, Melting in buffer I (0.05 M Na⁺ phosphate, 1 mM EDTA, pH = 7.0); *b*, Melting in buffer I with 0.85 mol coat protein added per mol RNA; *c*, Melting in buffer II (0.05 M cacodylic acid, 0.04 M Na⁺, 8 mM MgCl₂, pH = 7.0).

strong evidence for that structure. Magnetic resonance measurements as a function of temperature yield crucial supporting information on the base composition and nearest neighbour sequence of each helix, as described in an accompanying paper¹². The sum of these observations provides a virtually unambiguous model for the base-paired secondary structure of an RNA molecule in solution.

Our measurements differ from a conventional melting curve in that we resolve the time dependence of the melting process after a temperature perturbation. The data are obtained as oscilloscope traces^{11,13} which display both the time dependence and the total absorbance change. The latter can be plotted as a function of average cell temperature (average of initial and final values) for a constant temperature-jump size. The result (Fig. 2) is a derivative of the standard melting curve. The presence of two maxima in the derivative curves indicates that melting occurs in (at least) two steps, as would be expected for the secondary structure in Fig. 1.

TABLE 1 Stability of RNA secondary structure

	T_m in 0.05 M Na ⁺	T_m in 8 mM Mg ²⁺	T_m adjusted 1 M Na ⁺	ΔG (25° C)	ΔG (61° C)	ΔG (83° C)
Hairpin <i>b</i>	48° C	64° C	61° C	-5.0 kcalorie-mol ⁻¹	0 kcalorie-mol ⁻¹	+3.2 kcalorie-mol
Hairpin <i>a</i>	70° C	88° C	83° C	-11.4 kcalorie-mol ⁻¹	-4.5 kcalorie-mol ⁻¹	0 kcalorie-mol

The experimental T_m values were adjusted to 1 M Na⁺ by assuming an increase in T_m of 13° C for an increase by a factor of 20 in Na⁺ concentration. This increment is the average of the estimated minimum (8° C, from ref. 14) and maximum (17° C, in 8 mM Mg²⁺, Fig. 2c) variation. ΔG values refer to 1 M Na⁺.

The time resolution of the absorbance change indicates that each maximum in the differential curve corresponds to melting of a single helix, because the absorbance change separates into just two relaxation components. One of these is very fast, less than a few microseconds, and is nearly constant in amplitude between 40° C and 80° C. This is the usual behaviour for unstacking of bases in single-strand regions, and for temperature variation of the double helix absorbance^{11,13}. The other relaxation component is slower, can be described by a single exponential decay, and its amplitude peaks sharply at the maximum of the differential curve; relaxation times are shown in Fig. 3. These properties are characteristic of the melting of model double

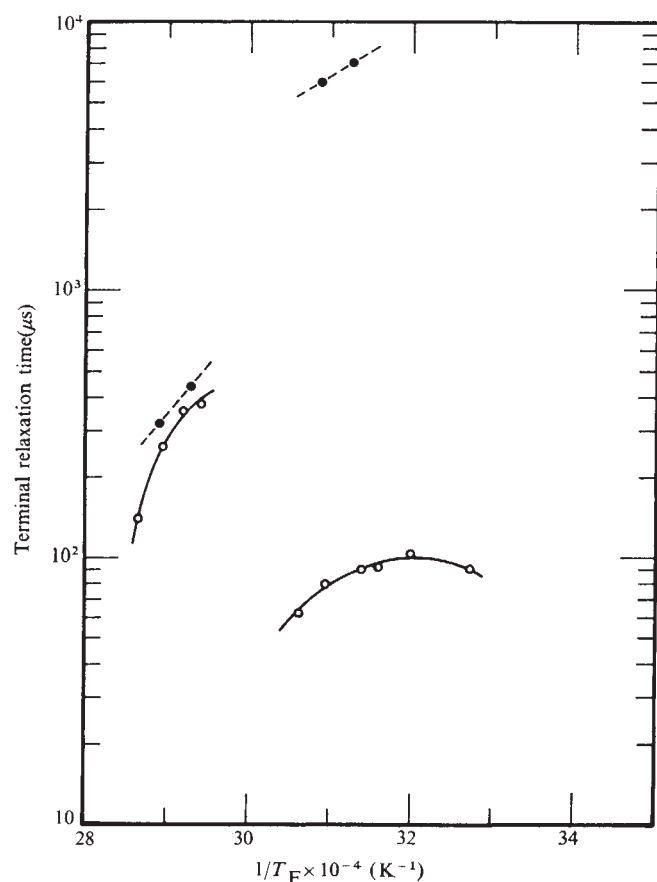


FIG. 3 Measured terminal relaxation time (τ), plotted logarithmically versus reciprocal of final temperature. Open circles were obtained in buffer I described in Fig. 2. All relaxation could be fit to a single exponential. Closed circles were obtained in the same buffer with added coat protein as described in Fig. 2b. Only four points are shown since the relaxations were difficult to analyse quantitatively over the entire temperature range. The data could be fitted to a single terminal exponential, but an additional exponential of approximately 100 μ s was also present in the lower temperature transition.

helices^{11,13-16}. If two double helices were melting at one of the differential maxima, they would have to have identical kinetic properties and identical T_m values at two salt concentrations in order to give a single relaxation component. Such a coincidence is sufficiently unlikely that it can be discounted.

The T_m and enthalpy of melting for each of the two helices can be extracted from the differential melting curve by methods described previously^{11,13}. The results are shown in Tables 1 and 2, where the heat and thermal stabilities are compared with values expected for helices *a* and *b*, based on our published thermodynamic parameters for model helices¹¹. Agreement of observation and prediction is good if the lower temperature transition is assigned to hairpin *b*. This assignment is strongly supported by the NMR results in an accompanying paper¹².

Table 1 also includes an estimate of the free energy of forming each helix at several temperatures, calculated from the measured heat of melting and the T_m corrected to 1 M Na⁺. At 25° C, secondary structure formation is strongly favoured; we estimate that the fraction of molecules in which helix *b* is unbonded at 25° C (1 M Na⁺) is about 2×10^{-4} and even less for helix *a*. Therefore we conclude that the RNA fragment forms a stable secondary structure, and the pattern of thermal unfolding is that expected for the two hairpin helices predicted from the sequence. We find no evidence for any additional stable structure with appreciable hypochromism.

Combination of the differential melting curve with the relaxation times allows calculation of forward and reverse rate constants for each helix^{11,13,15} (Fig. 4). The observed rate of helix formation is somewhat slower than is found for some model hairpin helices^{11,14}, but not unusual when compared with values found for tRNA^{17,17*}.

Binding of coat protein

When intact R17 RNA is incubated with coat protein, the formation of a complex can be detected by sedimentation in sucrose gradients²¹. Estimates of the number of protein molecules bound per RNA range from one to six^{18,21}. Since coat protein is a known inhibitor of replicase expression, it was suggested that the bound protein interferes with translation by directly preventing the attachment of ribosomes to the beginning of the replicase gene¹⁰. We have shown that the fragment protected by coat protein (Fig. 1) assumes a stable secondary structure, and we can now ask

TABLE 2 Comparison between predicted and experimental thermodynamic parameters

	Experiment		Prediction	
	T_m	ΔH	T_m	ΔH
Hairpin <i>b</i>	61° C	-47 kcalorie	59° C	-56 kcalorie
Hairpin <i>a</i>	83° C	-70 kcalorie	80° C	-80 kcalorie

Parameters refer to 1 M Na⁺.

whether the interaction with coat protein alters its physical properties.

First, we showed that the coat protein rebinds to the isolated fragment and determined the stoichiometry of the interaction. The apparent molecular weight of the complex as a function of added coat protein was measured by sedimentation equilibrium (Fig. 5). The weight average molecular weight of the RNA reaches a plateau value after the addition of 1 mol coat protein per mol RNA. Agreement between the observed molecular weight and the actual value (both the nucleotide and amino acid sequences are known) requires that the unknown effective partial specific volume ($\bar{v}$) of the complex be 0.74. The end point of the binding curve clearly indicates that on the average a 1:1 complex is formed, as has been reported previously for the whole RNA containing this sequence¹⁸.

Does the presence of bound protein affect the melting of the RNA secondary structure? Temperature-jump melting curves were obtained for the RNA-protein complex (Fig. 2b). The kinetics for the terminator helix *a* are unchanged, but strikingly, the rate of melting the initiator hairpin *b* is 100 times slower in the complex (Fig. 3). Coat protein therefore must interact with hairpin *b* in a manner which slows its melting. Although the terminator hairpin kinetics are unchanged, we cannot positively exclude the possibility of its interaction with coat protein since even the bound

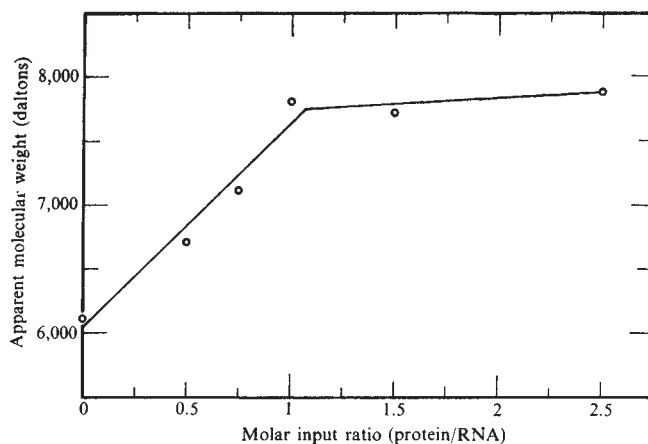


Fig. 5 Apparent molecular weight of the RNA-protein complex in buffer I as a function of added coat protein. Molecular weights were measured at 23° C and 266 nm in a model E analytical ultracentrifuge equipped with ultraviolet scanning optics. These were calculated in the standard manner, where M (apparent) = $(2RT/\omega^2)/(\partial \ln c/\partial x^2) = (1 - \bar{v}\rho)M$ (true), where $\bar{v}$ is an effective partial specific volume that also accounts for polyelectrolyte effects. Each point represents a run where previously unused RNA was mixed with coat protein at the indicated relative molar concentrations. The concentration of RNA was always 0.66 A ml⁻¹ at 260 nm. Plots of $\log c$ against x^2 were linear within experimental error. Under the conditions used, the absorbance is predominantly that of the RNA, so M (apparent) is only slightly affected by the presence of free protein.

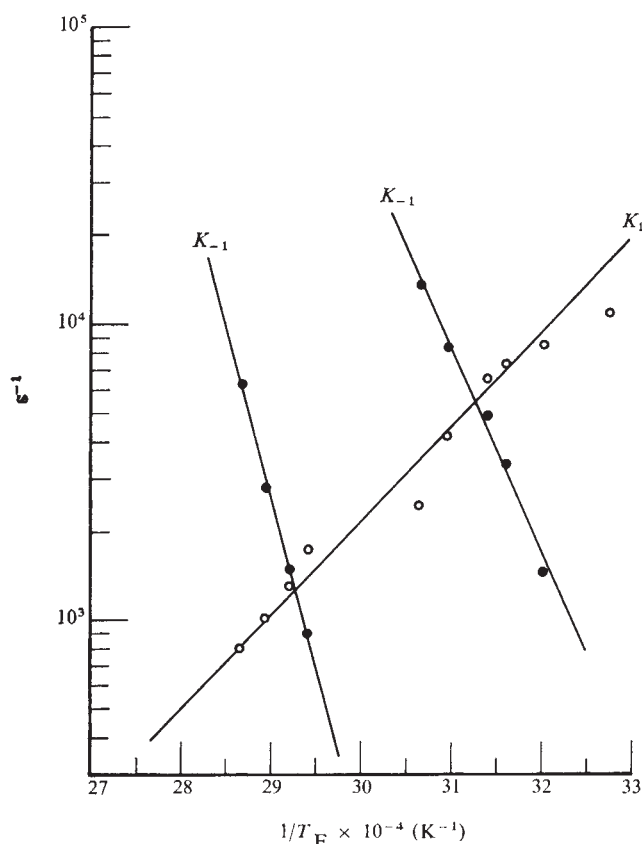


Fig. 4 Arrhenius plot of the temperature dependence of the RNA formation (k_1) and dissociation (k_{-1}) rate constants in buffer I. Resulting dissociation activation energies are +32 kcalorie for the lower transition and +54 kcalorie for the upper; the formation activation energy is -14 kcalorie in both cases. Rate constants were calculated as follows: the T_m and ΔH corresponding to each transition were determined¹³ from the peak position and width of the corrected differential melting curve (see Fig. 2), allowing calculation of the equilibrium constant K at any temperature. Knowledge of the relaxation times (Fig. 3) is then sufficient to calculate the rate constant by the equations $K = k_1/k_{-1}$ and $1/\tau = k_1 + k_{-1}$.

protein might become denatured at this higher melting temperature. The melting temperature (within experimental error) of neither hairpin is altered upon complex formation (Fig. 2b). This observation by itself could mean that the protein binds equally well to the hairpin helix *b* and the single strand, or that the free energy of binding is nearly zero at the T_m , or that an irreversible process, presumably involving protein denaturation, occurs at the T_m . We favour the last explanation because of the thermal instability of the isolated R17 coat protein.

What aspect of RNA structure is responsible for specific recognition of the fragment by R17 coat protein? We have found that (1) on the average one coat protein is bound per RNA molecule (Fig. 5); (2) the RNA is recognised while it assumes a stable secondary structure (Fig. 1); (3) the rate of melting of the helix which includes the initiator triplet is slowed in the RNA-protein complex (Fig. 3); (4) T_1 digestion of the R17 RNA-coat protein complex sometimes yields only the initiator hairpin in the protected fragment but never only the terminator hairpin (Fig. 1); (5) coat protein will not rebind the T_1 RNase-digested fragment¹⁹; (6) coat protein will specifically bind the isolated replicase initiator region¹⁹, which includes only hairpin *b* of the fragment in Fig. 1.

We conclude that coat protein recognition most likely requires some aspect of the RNA secondary structure, rather than simply a single-strand sequence. The structure which is recognised includes the ribosome binding site at the beginning of the replicase gene. Assuming that a similar structure is formed in the intact R17 RNA molecule, the direct interaction of coat protein with this region would provide a molecular explanation for the translational repression.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Are binary systems involved in supernova explosions always disrupted?

No known pulsar is a member of binary system. Does this mean, as has been suggested¹, that pulsars are the result of supernova explosions (hypothesis 1), and explosions which occurred in binary systems have in most cases disrupted the systems (hypothesis 2)? The discovery of two X-ray 'pulsars' (Cen X-3 and Her X-1) and other compact X-ray sources in binary systems seems to be an argument against hypothesis 2 if we believe that hypothesis 1 is also valid for compact X-ray sources. Here I show that hypothesis 2 is indeed untenable from the point of view of present theories of close binary evolution.

A binary system with period of several years may be close enough for the original primary to lose a considerable amount of its mass through the mass transfer in the late stage of its evolution². Van den Heuvel³ shows that 80% of the known OB spectroscopic binaries will undergo case B mass transfer (during hydrogen shell burning). Therefore it is reasonable to believe that supernova explosions in close binary systems are mostly preceded by mass transfer.

It is interesting that all known massive X-ray binaries have a common property that they consist of an OB supergiant with mass of about $30 M_{\odot}$ and a compact object with mass in the range between ~ 0.5 and $\sim 5 M_{\odot}$ (ref. 4). In five of the six known X-ray binaries the compact object has $M < 3 M_{\odot}$; only in Cyg X-1 might it be more massive. The large mass ratio of these massive X-ray binaries supports the hypothesis that the explosion is more likely to have occurred after the mass transfer. In that case the exploded star is more likely to be the less massive component⁵.

I assume that the mass of the original secondary (presently the primary) is constant during the explosion. The explosion is supposed to be spherically symmetric and to take place instantaneously. I also assume that initially the orbit is circular. Adopting these assumptions and taking the effects of the impact of the supernova shell onto the unexploded component into account in the way formulated by Colgate⁶, one can show that the condition for a binary

system to remain bound after a supernova explosion is⁷

$$\alpha + 2\beta - \gamma - 1 > 0, \quad (1)$$

where $\alpha = M_2/M_1$, $\beta = M_1'/M_1$ and γ is the ratio (gain in kinetic energy of the unexploded star due to the impact)/(initial orbital energy). M_2 is the mass of the unexploded component; M_1 and M_1' are the mass of the exploded star before and after the explosion respectively.

I assume a typical value for the velocity of matter ejected by a supernova explosion is 10^9 cm s^{-1} (ref. 8). For a given combination of values of M_2 , M_1' and the initial orbital period P , one can determine from the inequality (1) the critical value of α (α_{cr}) below or equal to which the condition (1) is unsatisfied (that is, the binary will be disrupted). I take $M_2 = 30 M_{\odot}$ and $M_1' = 1$ and $5 M_{\odot}$ re-

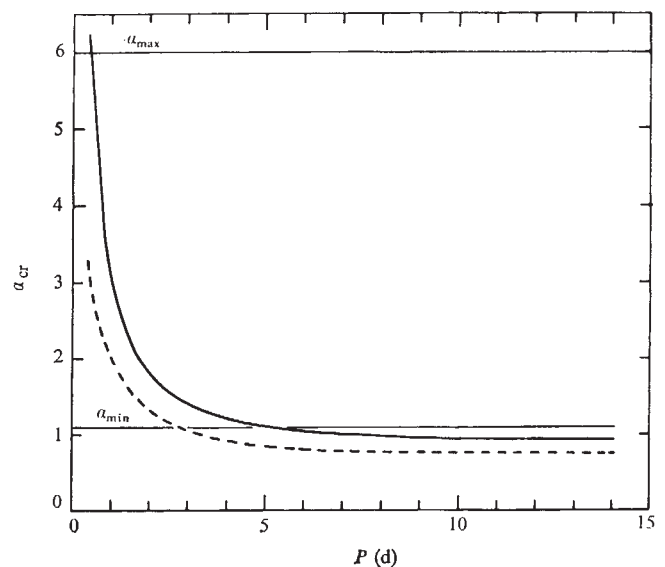


Fig. 1 The values of α_{cr} as a function of P for systems with final masses $30 + 1 M_{\odot}$ (—) and $30 + 5 M_{\odot}$ (---). For a given value of P the binary system will be disrupted if $\alpha < \alpha_{cr}$. The horizontal lines labelled α_{min} and α_{max} indicate respectively the lower and upper limit of the possible values of α (see text).

spectively. These values seem to be representatives for all massive X-ray binaries. The values of α_r as a function of P are given in Fig. 1.

It is likely that in massive X-ray binaries only a small fraction f of the transferred mass will escape from the system⁹. I assume that $f \lesssim 30\%$ which is consistent with the conclusions obtained from observations of evolved close binaries^{3,10}. If the explosion takes place after case B or case C mass transfer and if $f \lesssim 30\%$, the possible values of α are between ~ 1.1 and ~ 6 for $M_2 = 30 M_\odot$ (my unpublished work). From Fig. 1 one can see that all binaries with periods larger than about 5 d will remain bound after the explosion. Binaries with such periods form about 50% of the O-B5 spectroscopic binaries in Batten's Catalogue¹¹. Since, if the remnant mass is larger than $1 M_\odot$, also some binaries with a period of less than 5 d remain bound after the explosion, the probability of maintaining close binary systems (at least for close binaries with an O-B5 component) through supernova explosions should be considerably larger than 50%. Therefore the disruption of such binary systems involved in supernova explosions is more likely to be an exception rather than a rule.

Even if f is 40%, systems with $P \gtrsim 5$ d in which the remnant mass is $\gtrsim 2 M_\odot$ will remain bound. My results do not depend critically on the exact choice of a limit for f . So it seems necessary to seek another explanation for the non-existence of radio pulsars in binary systems. The absorption and smearing out of the radio pulses by the gas flows in the system might be such an alternative explanation.

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The equations determining the structure of a non-uniformly rotating star can be averaged over spheres and, provided the rotation is not too large, approximate to^{3,4}

$$\frac{1}{\rho} \frac{dP}{dr} = -\frac{GM_r}{r^2} \rho(1 - \alpha), \quad \alpha = \frac{2}{3} \left(\frac{\Omega^2 r^3}{GM_r} \right)$$

$$\frac{dM_r}{dr} = 4\pi r^2 \rho$$

where the symbols have their usual meaning.

The remaining stellar structure equations can be similarly averaged but are the same as the non-rotating equations to order α^2 . If α , the ratio of centrifugal force to gravity were constant throughout the star we could write $G^* = G(1 - \alpha)$ and the rotating structure equations are identical to the equations governing spherical star, in particular the relative variation of the physical variables is unchanged (that is T/T_c in terms of r/R for example), although their absolute value would be changed. Simple homology arguments then give

$$L \propto \frac{(\mu G)^8}{X^{1.2}}, \quad T_c \propto \frac{(\mu G)^2}{X^{0.4}}, \quad \mu = \frac{4}{(3 + 5X)}$$

where X is the fraction by mass of hydrogen; so that for $L = \text{constant} = L_\odot$, T_c changes negligibly and Σ_* is only very slightly increased. On the other hand, if the angular velocity is constant α increases outwards and by scaling the models of Faulkner, Roxburgh and Strittmatter⁴ we see that for fixed L , T_c increases and Σ_* increases more substantially.

These results, which are borne out by accurate calculations (to be published), suggest trying models where α decreases outwards; in such models the relative variation of the variables is changed—the temperature profile for example, is flattened, (Fig. 1). For such models we may expect a decrease in central temperature for a fixed luminosity and hence a decreased neutrino flux.

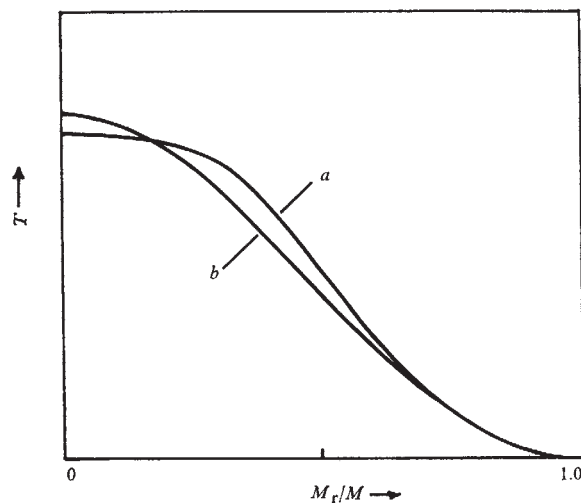


Fig. 1 The effect of non-uniform rotation on the temperature distribution inside stars. a , $\Omega^2 r^3/GM_r$ decreases outwards; b , $\Omega = 0$.

Internal rotation of the Sun and the solar neutrino flux

THE low upper limit of 1 SNU (10^{-36} captures per ^{37}Cl atom s^{-1}) on the observed neutrino flux from the Sun obtained by Davis¹ has proved an embarrassment to stellar physicists, and in spite of considerable intellectual gymnastics the standard solar models predict at least 6 SNU (ref. 2). The essential difficulty has been to produce a model with a low enough central temperature that can still produce the observed luminosity of the Sun with an age of 4.7×10^9 yr.

Arguments for a rapid central rotation, causing a low neutrino flux, are elaborated below.

The Sun, of course, has evolved for some 4.7×10^9 yr and in inhomogeneous evolved models there are two factors lowering the neutrino flux compared with the standard models: first, the change in the temperature profile demonstrated previously; second, the increased homogeneity as compared to models with $\alpha = 0$ due to the increased region of nuclear burning caused by the changed temperature gradient. Detailed calculations show that these effects can reduce the

neutrino flux for the present Sun to less than 1 SNU (Table 1).

TABLE 1 Inhomogeneous solar model ($t = 4.7 \times 10^9$ yr; $L = L_Z/X = 0.019$)

	X_c	$\log T_c$	$\Sigma\phi$
$\alpha = 0$	0.35	7.17	6.5
$\alpha = \alpha^*$	0.50	7.11	0.93

(see Fig. 3)

In passing I note that it is no surprise that the calculation by Ulrich⁵ gave no substantial decrease in the neutrino flux. In his model Ω was constant and therefore α increased outwards in a core. It then dropped discontinuously to zero. We would not expect the right change in temperature profile from such a model.

The angular momentum per unit mass of interstellar gas clouds is so large that if a star forms it is highly probably that it will be rapidly rotating. Subsequent contraction exacerbates the problem; the central regions would spinup leading either to dynamic instabilities and possibly fission⁶

or just a dynamically stable rapidly spinning core. Detailed calculations of contracting solar models including the effect of rotation have been made by Williams⁷ and in Fig. 2 I show the ratio of centrifugal force to gravity, α , after contraction. This angular velocity distribution is not stable to thermal perturbations, that is, those where radiative gains compensate for the stable stratification so that the fluid responds like an incompressible liquid^{8,9}. The instabilities on a scale l have growth rate given by

$$\tau = \tau_{th}(l/R)^2 1/\alpha, \quad \tau_{th} = 3GM^2/(4RL)$$

where τ_{th} is the thermal response time of the star $\sim 2 \times 10^7$ y. Since the Reynolds number is so large we must expect these instabilities to be turbulent and we can then estimate the mixing time by assuming a random walk process, requiring $(R/l)^2$ steps of length l . The mixing time is then the same for all models and is τ_{th}/α or about 10^8 yr.

During this time the chemical composition has changed due to the central burning of nuclear fuel, so there is now a chemical gradient which can stabilise an angular velocity gradient.

The angular velocity therefore adjusts initially to a marginally stable state in one or two mixing times and then continually readjusts as the Sun is slowed down by angular momentum loss in the solar wind. In the first stage there has to be substantial mass loss of about $0.3 M_\odot$. The results of detailed calculations are given in Fig. 3; the centre of the Sun is left spinning rapidly while the bulk is slowed down, α decreases outwards and this gives the low neutrino flux.

The external quadrupole moment has a value of about $J_2 = 6 \times 10^{-8}$, comparable with Dicke's measured value. The central region spins at a phenomenal rate with a period of about 50 min—it would be of considerable interest to look

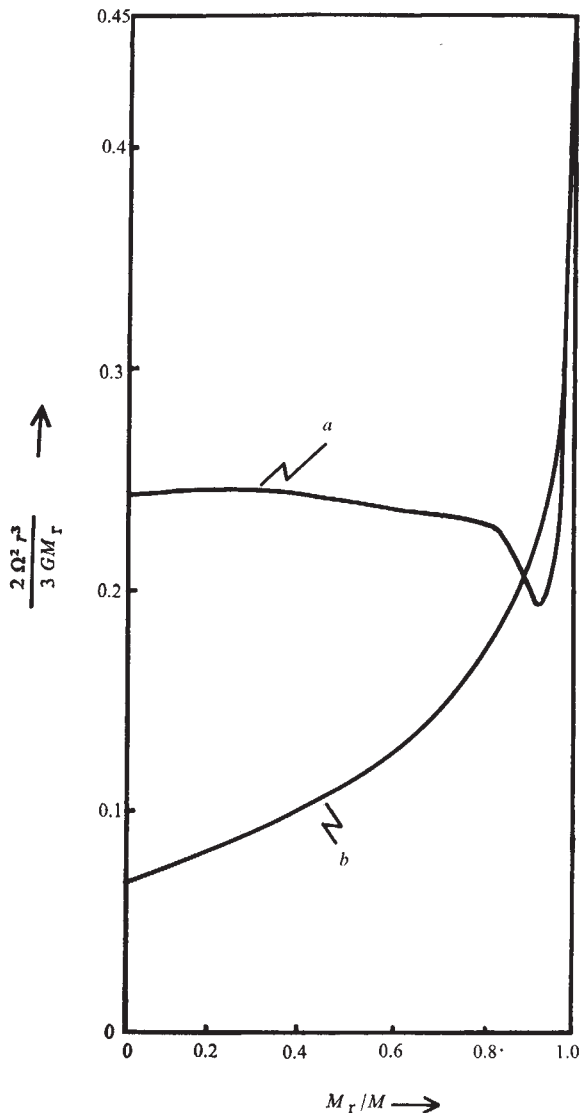


FIG. 2 Ratio of centrifugal force to gravity in a solar model before and after pre-main sequence contraction. *a*, End of contraction; *b*, Beginning of contraction.

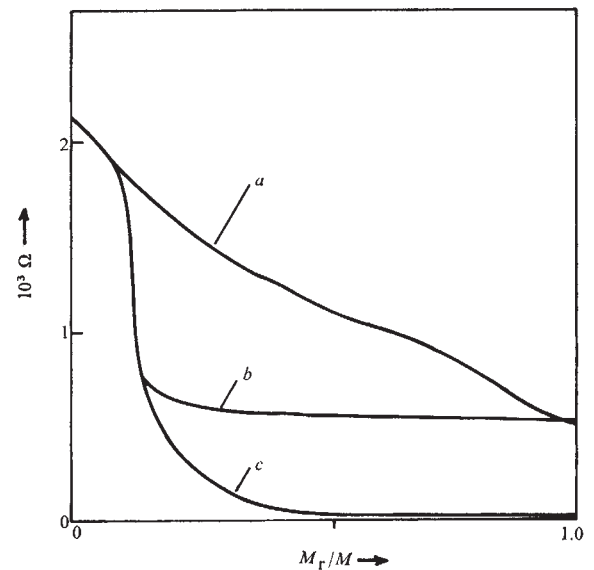


FIG. 3 Angular velocity inside the Sun: *a*, after contraction; *b*, after re-adjustment; *c*, after solar wind braking. *c* is the distribution Ω^* used in the text.

for phenomena on the Sun with a period of 30 min to 1 h that might reflect this rapid motion; if it exists it might lend support to the "rapid core" explanation of the low neutrino flux.

Care should, however, be exercised before concluding that these models support the scalar tensor theories of gravity. In those theories the constant of gravity decreases in time which accelerates the nuclear burning and so increases the central temperature increasing the neutrino flux.

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Light curves of very faint meteors

THE variation of brightness as a function of height when a meteoroid burns up (ablates) in the Earth's atmosphere is governed by the physical properties of the meteoroid. The basic theory of the ablation of a solid meteoroid to due to Öpik¹, and the theoretical light curves are in fairly good agreement with the observed light-curves of very bright meteors². In contrast to this the observations of Jacchia³ and Hawkins and Southworth⁴ of faint photographic meteors show that the train lengths of these meteors are much shorter than predicted by the classical theory, and it is principally due to evidence of this sort that the "dustball" theory of Whipple⁵ which supposes the original meteoroid to consist of an aggregate of many small solid particles, has become very popular. Nevertheless Jones and Kaiser⁶ found that by extending the classical ablation theory to include the effects of the meteoroid's thermal capacity, conduction and radiation they could explain many features of the experimental data in terms of compact meteoroids which fragment as a result of thermal shock. According to Jones and Kaiser⁶ it is possible to choose between the two theories on the basis of observations of very faint meteors (magnitude $M > +3$) since these meteoroids should not fragment due to thermal shock if they are compact; on the other hand if they are fragile conglomerates they will probably continue to show the effects of severe disintegration.

To this end we have developed an observing system⁷ using sensitive television equipment which has a limiting sensitivity of $+8.5$ mag for stars when operating with an $f/0.95$, 50 mm lens.

On the night of September 31/October 1, 1973 in London, Ontario, 179 meteors were observed with the system between 11 p.m. and 6 a.m. with the camera pointing north at an elevation of 50° .

Analysis of the video tapes enabled the train lengths perpendicular to the line of sight to be determined assuming the meteors to occur at an altitude of 95 km. The apparent train length l_a is related to the height interval between the starting and finishing height dh by the relation

$$dh = rl_a$$

where r is a random variable depending on the geometry of the camera and the meteor trains. Since at that time of year the activity of meteor showers is expected to be low, we tried two assumptions in order to estimate the mean value of r ; a random distribution of radiant and also a point radiant such that all the meteors were supposed to come from the apex of the Earth's way. Fortunately each of these assumptions yielded a mean value of r very close to unity.

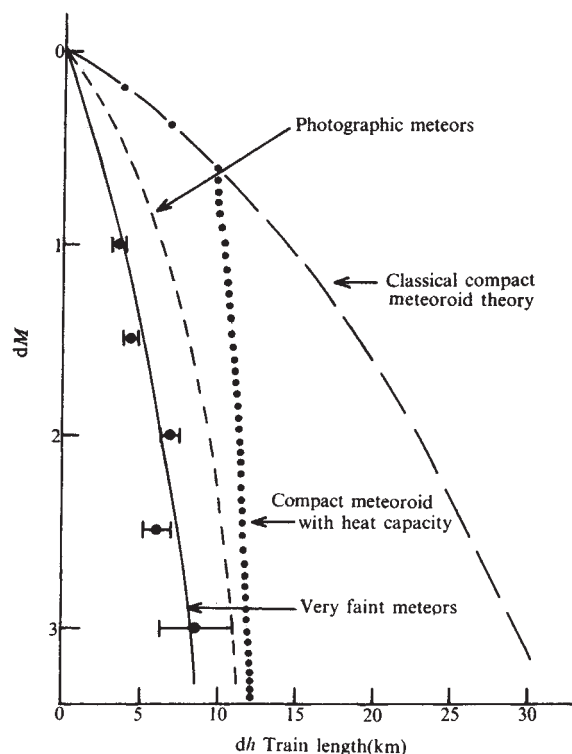


FIG. 1. The difference dM between the magnitude of the meteors and the limiting magnitude of the observing system plotted against dh , the height interval between the starting and finishing height of the meteors.

In this way we obtained the results in Fig. 1 which shows dh as a function of dM , the difference between the magnitude of the meteor and the limiting magnitude of the system. For the sake of comparison we have also shown the theoretical predictions for the compact meteoroid model and the curve of dh against dM computed using the photographic data of Hawkins and Southworth.

It is clear that not only are the train lengths of the television meteors considerably shorter than predicted by the compact meteoroid model but they are also significantly shorter than those of the photographic meteors. We interpret this as indicating that fragmentation is even more severe for the very faint meteors than for the meteors of Hawkins and Southworth observed using Super-Schmidt cameras. On the basis of these results we conclude, albeit reluctantly, that the compact meteoroid model is no longer tenable.

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Strong long-period tropospheric and stratospheric rhythm in the Southern Hemisphere

THE recent EOLE satellite-balloon atmospheric sensing experiment conducted in the Southern Hemisphere between August 1971 and July 1972, provided large quantities of upper tropospheric data in a hitherto near-dataless region. Besides measurements of ambient temperature and pressure reported directly from the balloon to the satellite, the experiment allowed the calculation of series of highly accurate Lagrangian velocities by the positioning of the balloon between successive satellite orbits. The experiment and data are described in detail by Morel and Bandede¹.

To obtain a Eulerian data set, the Lagrangian data required transformation. As no general analytical transformation exists², the Eulerian information was obtained by computing daily averages of all the measured Lagrangian quantities in each area of 10° latitude by 20° longitude. The resulting average was assumed to be the representative Eulerian daily average of that quantity. The rationale and justification of this method is given elsewhere (ref. 3 and P.J.W., D. G. Curtin and Y. Mintz, to be published).

Tropospheric rhythms: The many attempts to find a distinct atmospheric periodicity on a global or hemispheric scale have met with little success. Most notable are the efforts of Willett⁴ and Namias⁵ in the Northern Hemisphere and recently by Taljaard⁶ in the Southern Hemisphere. All studies defined some index which was more or less sensitive to the change of state of the atmosphere. Although large variations in the various indices did occur, no dominant rhythm was identified⁶.

Another common feature of these studies was the use of land based Eulerian sensors which, on account of their geographical grouping, may not have been suitable for the calculation of a representative hemispheric index. To overcome this problem, the Eulerian data inferred from the dense EOLE data set was used to define a zonal index sensitive to the transition of the atmosphere from perturbed to a zonal state. Such an index (R) is defined as the ratio of the kinetic energy of the mean flow (K_z) and the zonal average of the perturbation or eddy kinetic energy (K_E), that is,

$$R = K_z / K_E = \frac{1}{2 \cos \phi} \int_0^{2\pi} (\bar{V} - [\bar{V}])^2 d\lambda / ([\bar{V}]^2 / 2)$$

where

$$[\bar{V}] = \frac{1}{\cos \phi} \int_0^{2\pi} \bar{V} d\lambda.$$

Here λ represents longitude, ϕ latitude and $\bar{V}$ the distribution of the daily mean Eulerian velocity inferred from the EOLE data.

Time sections of R are shown in Fig. 1 for three 10° latitude bands between 30° and 60°S. Large amplitude variations may be seen with an apparent period of some 20 d, especially in the higher latitudes. These represent regular changes from highly perturbed states (large R) to highly zonal states (small R). Superimposed upon this trend are variations of higher frequency and smaller amplitude indicating similar but short lived variations.

The times series spectral analyses of $R(t)$, shown in Fig. 2, tend to support these observations. In the 50°–60°S latitude spectra for R , a strong isolated peak suggests a strong rhythm centered near 20 d in the high latitudes, a feature which is also reflected in the K_E spectra. As illustrated by the 40°–50° spectra of R , the peak is somewhat weaker and further diminishes into the subtropics. Spectra of the zonally averaged pressure (lower diagram) indicates a weaker periodicity near 20 d, whereas the zonally aver-

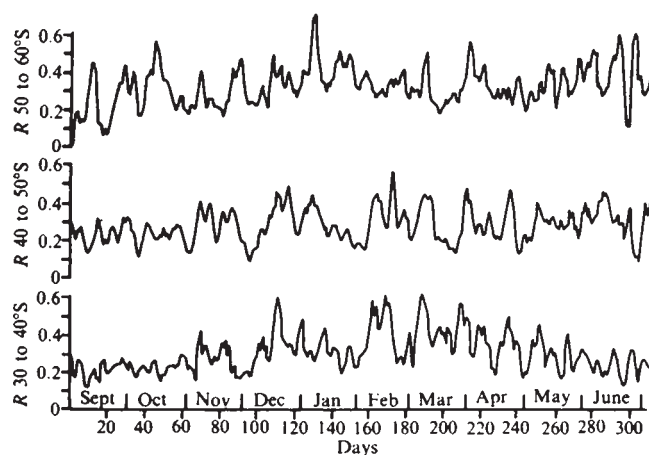


FIG. 1 Variation with time of the index R over the EOLE period for the three latitude bands indicated.

aged temperature field does not exhibit relatively as large a variation. This is common to the temperature field at all latitudes.

The cross spectra between the two component energies of $R(t)$ indicate an extreme peak near 20 d. An important feature is that, in the 50° to 60° band, K_E and K_z are almost exactly out of phase (-176°) and have a coherence of 0.825 in the 18 to 23 d band, surpassing the 99.9% confidence limit at this spectral band width. (Coherence between the various values of R for the different latitudes each

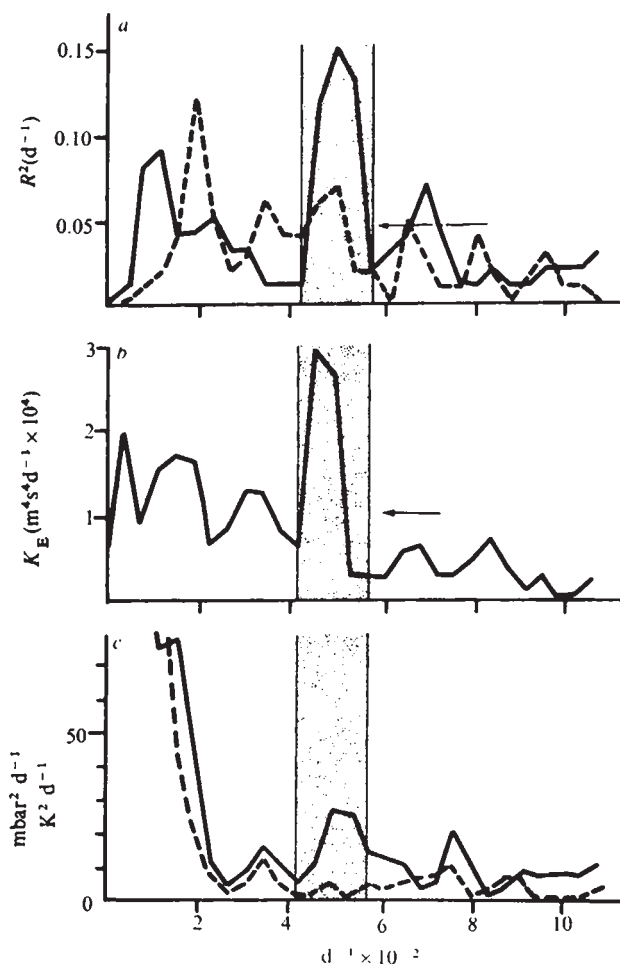


FIG. 2 Variance spectra. a: —, R for 50° to 60°S; ---, R for 40° to 50°S. b: K_E for 50° to 60°S. c: —, P ; ---, T . Both for 50° to 60°S. Arrows denote 90% confidence level, shaded region the 18 to 23 d band.

surpasses the 99% level in this frequency band.) Such phase and coherence strongly suggests that the quasi-20-d rhythm in R may be accounted for by the conversion of kinetic energy between that of the eddies and that of the mean flow, such that the cycle consists of waves growing at the expense of the mean flow and then the reverse conversion.

Spectral analysis in space and time indicates that much of the power of the 20-d rhythm is in the ultra-large scale waves. For example, in the 50° to 60° band, the kinetic energy in longitudinal wave numbers 1 and 2 is an order of magnitude greater in the 18 to 23 d period interval than for any other wavenumber. The momentum flux also shows a 20-d rhythm, which, together with the above, suggests that this variability in R is a manifestation of a slow period and very large scale barotropic energy conversion between K_z and K_x which is similar in character to the barotropic energy conversion described by Lorenz⁷. But this process is generally thought to be restricted to a much smaller scale ($\sim 1,000$ km) and higher frequency motions (~ 4 to 5 d) and is considered to be of secondary importance in the energetics of the general circulation.

In contrast, we suggest that the precominant process in upper-troposphere of high latitudes over the time scale of weeks is the barotropic exchange of energy between the ultra-long waves and the zonal flow.

(ii) Stratospheric rhythms: Initial attempts have been made to determine to what extent the stratosphere is effected

by the large scale, long period tropospheric oscillations suggested above. Due to the scarcity of conventional data and the consequent inability to construct a zonal index for the stratosphere, total ozone data from the few scattered Southern Hemisphere stations were analysed.

Figure 3 shows the time series spectral analyses of the total ozone for the three stations, Argentine Island (65°S), MacQuarie Island (55°S) and Brisbane (28°S) chosen for the initial study on the basis of data availability and latitudinal spread. The only data modification was the removal of the dominating annual trend before the spectral analysis.

The common feature of all three spectra is the existence of power, to varying degrees, in the 18 to 23 d period band. This is especially evident at Argentine Island and MacQuarie Island, and surprisingly for a station so far equatorward, at Brisbane. Cross spectra between these ozone stations show strong coherence during the EOLE period in the 18 to 23 d band. The coherence between R and the stratospheric ozone is slightly weaker but this may be because we are seeking cross-spectra between point observations (the ozone) and hemispheric quantities (R).

The results presented here are not conclusive, but there seems to be a strong suggestion of a preferred and dominating physical mechanism existing over the period of weeks in the high latitudes of the upper troposphere of the Southern Hemisphere. This large scale and long period oscillation seems to be tied to the barotropic interchange of energy between the perturbations and the mean flow and possesses vertical propagation properties such that it may provide an important energy source for the stratosphere over this time scale.

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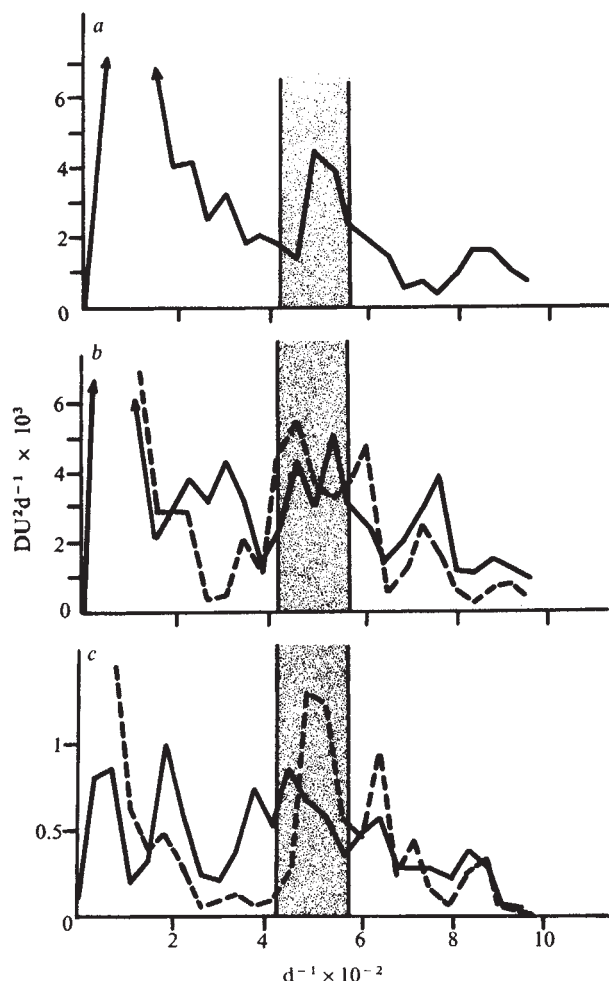


FIG. 3 Variance spectra of total ozone. *a*: Argentine Island (65°S), 1962-64. *b*: MacQuarie Island (55°S), 1971-72. *c*: Brisbane (28°S), 1971-72. Dashed lines denote data from EOLE period. 1 DU is equivalent to 10^{-3} cm NTP.

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Dispersion of ionospheric waves

THE phase speed dispersion of travelling ionospheric disturbances (TIDs) has been measured using data from a three-dimensional array of c.w. Doppler sounders¹. Horizontal and vertical trace speeds of phase surfaces were measured as a function of wave period by performing cross-spectral analysis of the array signals. Although the measured dispersion varied considerably depending on the coherence of the wave activity across the array, the average horizontal trace speed was approximately proportional to $T^{-1/2}$, where T is the period. From a very limited set of measurements, vertical trace speed was tentatively determined as proportional to T^{-1} . Additional measurements of highly coherent waves now indicate that the vertical dispersion is even stronger than T^{-1} at the short periods (12-25 min.).

Based on the reliably determined horizontal dispersion and a large diurnal variation of speed, I suggested¹ an

explanation of the observations in terms of hydromagnetic waves. This explanation did not account for the magnitude of the observed speeds but explained the horizontal dispersion and large diurnal variation of speed. Because of the difficulty of developing a satisfactory hydromagnetic wave explanation, I attempt here to show how the dispersion could be accounted for in terms of the widely accepted internal gravity wave explanation of TIDs (ref. 2).

The horizontal of phase speed gravity waves is a function not only of period but also of the tilt of the phase surfaces away from the vertical:

$$V_x = \frac{c}{\omega_0} [N^2 - \omega^2(1 + \tan^2 \phi(\omega))]^{1/2}$$

where N , ω_0 and ω are the Brunt, acoustic cutoff and wave frequencies, c is the speed of sound, and $\phi(\omega)$ is the tilt of the phase surfaces away from the vertical¹. As the phase surfaces tilt over towards the theoretical limiting angles of wavefront tilt, given by

$$\phi_L = \tan^{-1} \left[\frac{N^2}{\omega^2} - 1 \right]^{1/2}$$

the horizontal speed, V_x , approaches zero. When ϕ is zero (no tilt), or when ϕ is a fixed percentage of the limiting angle, V_x increases toward long periods. If, however, a wavefront tilt function, $\phi(\omega)$, is chosen in which phase surfaces tilt over closer to the limiting angles at long periods than at short periods, V_x can be made to decrease towards long periods. That is, the first equation above can be solved for $\phi(\omega)$ giving:

$$\phi(\omega) = \tan^{-1} \left[\frac{N^2}{\omega^2} - 1 - \frac{\omega_0^2 V_x^2}{c^2 \omega^2} \right]$$

For any specified horizontal phase speed dispersion, $\phi(\omega)$ will be less than ϕ_L and both will increase toward long periods with $\phi(\omega)$ approaching ϕ_L if V_x decreases toward long periods.

Figure 1 shows new observations of the mean horizontal and vertical phase speeds of ionospheric waves recorded between 1700 and 2200 LT on four successive days (November 22-25, 1972) in the New York City area. The horizontal speeds were determined from an array of four Doppler sounders operating at 4.5 MHz. The vertical speeds were determined from the phase lags of the downward-moving wavefronts between the reflection levels of 4.5 MHz and 2.5 MHz Doppler sounders. The absolute values of the vertical speeds are difficult to measure accurately because the difference in the reflection level of the two radio frequencies varies over the recording interval of 5 h. An average vertical separation of 20 km was however estimated using mean monthly electron density profiles for November 1972, at Wallops Island, Virginia, which is several hundred kilometers south of the array.

Both horizontal and vertical speeds decrease toward long periods. The observed phase speed dispersion can be best approximated by gravity wave theory if it is assumed that the waves propagate with the wavefront tilt given by the dotted line at the bottom of fig. 1. A Brunt period of 8 min was assumed which gives the limiting angle of tilt, ϕ_L , in fig. 1. The theoretical V_x and ϕ determine the theoretical V_z since $V_z = V_x / \tan \phi$. The theoretical speed decreases towards long periods because the theoretical value of ϕ approaches ϕ_L at long periods. The observed wavefront tilt is also shown. It does not entirely match the theoretical tilt function, but as required, is closer to the limiting angle at long periods. Either the observed V_x or V_z could be approximated more closely by a different theoretical tilt function but the best approximation to both is shown.

For the time of day of the observations, the radio signals from the Doppler sounders should have reflected near 200 km height, where the Brunt period is about 14 min for a

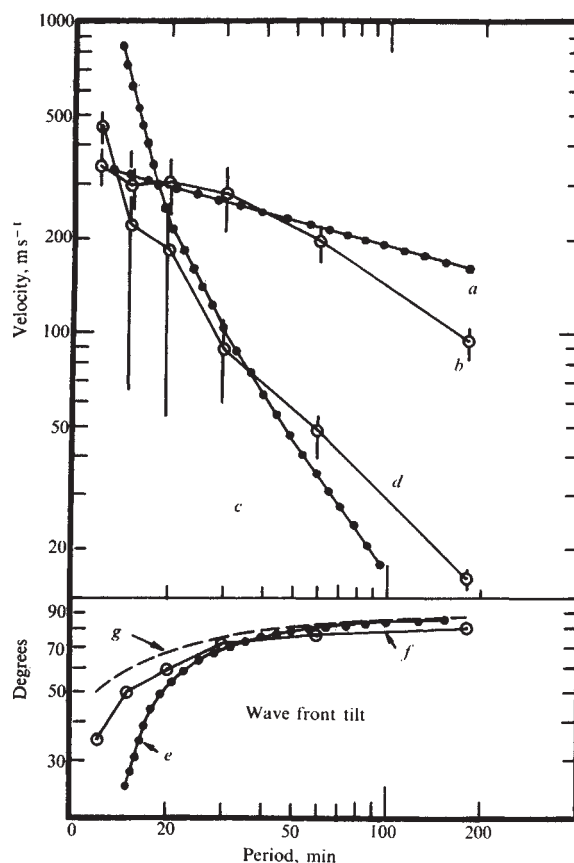


FIG. 1 Mean horizontal and vertical phase speeds and tilt angles of ionospheric waves recorded between 1700 and 2200 h LT on four successive days (November 22-25, 1972). Standard deviation bars are shown. Theoretical speeds and tilt function, consistent with gravity wave theory when the limiting tilt angle ϕ_L is assumed, are also shown. *a*, V_x theoretical; *b*, V_x observed; *c*, V_z theoretical; *d*, V_z observed; *e*, ϕ , theoretical; *f*, ϕ observed; *g*, limiting tilt angle.

1962 US Standard Atmosphere. The best fit of theoretical and observed curves was however, found using atmospheric parameters (N , ω_0 , c) that apply to about 125 km.

Theoretically, free interval gravity waves tend to propagate energy at increasing angles ($90^\circ - \phi_L$) about the horizontal as period decreases³. If however, a broad spectrum of waves from a single source were observed at a receiving point, then the short period waves would necessarily have travelled at lower percentages of their limiting angles than did the long period waves, thus producing the apparent inverse dispersion. Figure 1 indicates that over a wide range of periods, the wavefront tilts need be only a few degrees less than the limiting angles to produce the observed dispersion.

A large diurnal variation of the phase speed of the F-region waves has been reported⁴. The speeds increase hundreds of per cent from day to night, maintaining the apparent inverse dispersion. Although it is difficult to explain why, I suggest that for some reason the waves simply propagate at angles further from the limiting angles at night, causing larger phase speeds at all periods.

It would be useful to use a very widely spaced array and attempt to measure group speeds of a sufficient number of waves to detect group speed dispersion and any diurnal variation of group speed. Such measurements are, however, probably much more difficult than accurate phase speed measurements. According to gravity wave theory, horizontal group speeds must be less than phase speeds. My suggestion¹ that the observed horizontal dispersion implies a group speed greater than the phase speed¹, would be invalid for the strongly anisotropic behaviour of gravity waves. As indicated here, the apparent inverse phase-speed dispersion could result

solely from the behaviour of the wavefront tilt and thus the slope of the phase-speed dispersion curve would give no information about group speeds.

Brownlie *et al.*⁵ have reported ionospheric wave measurements which suggest higher phase speeds at the longer periods, and they noted the discrepancy with the observations of Herron¹, such as shown in fig. 1. The speeds, however, vary considerably with time of day⁴, and if care is not taken to eliminate this effect, it could distort the dependence on period.

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Will another damaging earthquake occur in Koyna?

AN earthquake of magnitude 5.1 (Richter scale) occurred on October 17, 1973, near the Koyna Dam (17°23'N, 73°45'E) which experienced a damaging earthquake¹ on December 10, 1967. The former earthquake was preceded and followed by several foreshocks and aftershocks. Two of the foreshocks on the same day had magnitudes of the order of 4.0 and there was another significant earthquake of magnitude 4.6 in the same region on October 24.

It is now well known that filling of large artificial lakes behind high dams can trigger earthquakes²⁻⁴. The examples of Kariba, Kremasta and Koyna are particularly significant since damaging earthquakes of magnitude exceeding 6 have occurred in these regions.

Before the construction of the Koyna Dam in the western part of the Peninsular Shield of India no significant tremor had been recorded. Soon after the impounding of the Koyna reservoir in 1962, reports of earth tremors near the dam site began to accumulate. Thereafter thousands of tremors were located within 25 km of the dam by a network of four closely spaced seismological observatories⁵. Up to June 1969, 450 of these shocks had magnitude ≥ 3 . The earthquake of September 13, 1967 (magnitude 5.5) was the

first significant event and caused mild damage in Koyna Township. The earthquake of December 10, 1967 had a magnitude of 6.0, claimed about 200 lives and caused widespread damage. The earthquake of October 17, 1973 (magnitude >5) occurred in the Koyna region after a lapse of 5 yr, the last one having occurred on October 29, 1968. An analysis of the records of the Hyderabad seismograph station, situated about 490 km east of the Koyna Dam, shows that the frequency of earthquakes of magnitude ≥ 4.0 has decreased during the past few years following that of December 10, 1967. Table 1 shows that nine such earthquakes occurred in 1969, seven in 1970, four in 1971 and three in 1972. Between January and September 1973 only one earthquake of magnitude 4.1 occurred (on April 19). During October 1973 three earthquakes of magnitude >4 occurred in addition to the one of magnitude 5.1. The present activity does not fit the trend of gradually decaying seismicity in the Koyna region during the past few years.

From studies of reservoir levels and earthquake frequency at several places⁴ it has been inferred that, in addition to the tectonic setting and geological conditions, the factors affecting the tremor frequency near the reservoirs include (1) the rate of increase of water level; (2) the duration of loading; (3) the maximum level reached; and (4) the period for which high levels are retained. These observations are supported by a statistical analysis of the water level and earthquake frequency for the Lake Mead⁶, Koyna,

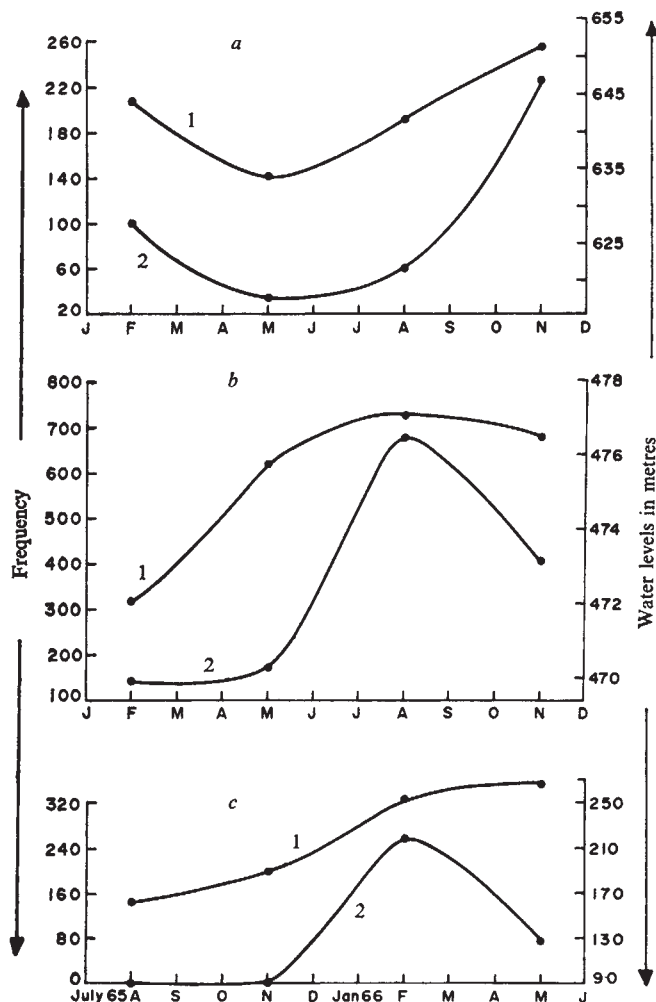


FIG. 1 Three-monthly average of water levels and total numbers of earthquakes for the same months at Koyna (a), Kariba (b) and Kremasta (c) for which the correlation coefficients are +0.93, +0.74 and +0.69, respectively. The periods include 1964 to 1968 for Koyna, 1959 to 1968 for Kariba and July 1965 to June 1966 for Kremasta. 1. Water level; 2, frequency.

TABLE 1 Koyna events of magnitude ≥ 4 since 1969

Date	Magnitude	Date	Magnitude
January 21, 1969	4.1	September 25, 1970	4.6
February 13, 1969	4.2	September 26, 1970	4.6
March 7, 1969	4.4	January 23, 1971	4.2
June 3, 1969	4.2	February 14, 1971	4.0
June 27, 1969	4.5	August 10, 1971	4.0
July 22, 1969	4.0	August 10, 1971	4.3
November 3, 1969	4.1	May 1, 1972	4.2
November 4, 1969	4.2	May 11, 1972	4.5
April 16, 1970	4.0	November 11, 1972	4.1
May 27, 1970	4.8	April 19, 1973	4.1
June 8, 1970	4.1	October 17, 1973	4.0
June 17, 1970	4.1	October 17, 1973	4.1
September 21, 1970	4.0	October 17, 1973	5.1
		October 24, 1973	4.6

Kariba and Kremasta reservoirs. In Fig. 1, for the latter three cases, water levels are given as averages over 3 months and the earthquake frequency as the total number of earthquakes registered in the same months for the whole of the period considered. The correlation between water level and frequency is readily apparent. Using other averages and monthly combinations, however, the correlation is less apparent and the high activity before and after the main shock greatly affects the frequency curve. Among the many examples of reservoirs with associated seismicity Koyna is the most outstanding; earthquakes occur whenever high levels are reached following rapid filling and are retained for long times. The highest water levels were maintained for longest⁷ during August to December 1967, corresponding well with the maximum seismic activity and the earthquake of magnitude 6.0 on December 10, 1967. The second highest level was reached during August to October 1965, which corresponds well with the second most conspicuous period of activity (during November 1965).

Reservoir levels in the Koyna Lake have been kept relatively much lower since December 10, 1967. No major earthquakes have occurred since October 1968 and this year water has again been allowed to accumulate in the reservoir, which was filled completely for the first time on August 15, 1973. The consequent high seismic activity included an earthquake of magnitude >5 and very strongly supports the idea of a persistent relationship between water level and earthquake activity.

The earthquake of October 17 is similar to that of September 13, 1967. The two earthquakes had comparable magnitudes, originated at the same place, had a similar foreshock-aftershock pattern and occurred after rapid filling of the reservoir to peak levels. Although it cannot be said with certainty that the present seismic activity heralds a strong earthquake in the Koyna region, there is a definite enhancement in the seismic activity which correlates with the increased reservoir levels.

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alies and concentration gradients that must exist in an ocean that continues to transport caesium from its terrestrial rocky sources to the seafloor.

Less is known about the long term steady state of progress of the natural non-radioactive nuclide ^{133}Cs through the ocean, than is known about the progress of the radioactive nuclides of caesium that have entered as nuclear fallout during the last decade. It would be reassuring to understand, for instance, how fast the surface layers of the ocean might dissipate the relatively larger amounts of ^{137}Cs that may enter as a consequence of the use of fissionable fuels.

Recently, analytical equipment specialized for traces of natural caesium was completed at the Scripps Institution² and tested with carefully collected North Pacific water samples. These measurements seem to demonstrate for the first time a significant difference between the caesium concentrations at the sea surface and in the deeper water. They also emphasise the uniformity of caesium concentrations in deep Pacific water masses, and the extreme similarity of caesium concentrations, normalised against salinity, at Pacific surface stations thousands of miles apart.

In essence, the new procedure is a computerised version of emission flame spectrometry, used after relatively large (2 l) samples have been concentrated by multiple extractions using the methods of Feldman and Rains³. Standard amounts (spikes) of caesium are added to alternate halves of each unknown and each reference sample, which are extracted in special gang tumblers⁴. A new form of sample changer which uses an automated distributing valve² burns the unknown spiked and unspiked concentrates, as well as the spiked and unspiked concentrates of 'reference standard seawater samples' in uniform succession. Burning of each of the four concentrates is done on six portions alternatively, and is programmed by a digital system tied into a desktop computer (Wang 600).

This allows standardising assays (on 10 ml organic concentrates containing $0.3\text{ }\mu\text{g}$ caesium) to be replicated with standard deviations $< 0.3\%$. At present, the standard error of replicating the complete comparison between two seawater samples is somewhat more, about 0.5% . Two to eight replications are, however, made.

Figure 1 summarises concentrations and positions at six North Pacific stations. Caesium determinations are normalised to a salinity of 35.00 parts per thousand and the bars give the standard deviation of the mean of assays for each sample. In August 1973 at stations 1 and 2 small (4 l) samples were collected at depths of 0, 1,000, 2,000, and 4,000 m. At station 1, a large (50 l) poly carboy of surface water was collected for tentative reference. Large samples were also collected at stations C and D, about 2,000 miles apart. The normalised means of caesium concentration for these three large recent samples differed little, 0.0005 (0.2%), from a mean value of $0.2990\text{ }\mu\text{g kg}^{-1}$.

The (large) 3-year-old samples, A and B, had concentrations departing from this mean less than might be expected from (wall) losses, and the two smaller surface samples, 1 and 2, differed only as predicted statistically.

Figure 1 indicates a downward decrease of natural concentrations of caesium in the open North Pacific Ocean. It is too early to discuss the differences apparent between 1,000 m and 4,000 m, but the mean of all (12) subsurface analyses of the six samples collected at 1,000 m or below is $0.295\text{ }\mu\text{g kg}^{-1}$ and the difference between this and the surface mean is $0.004\text{ }\mu\text{g kg}^{-1}$ (1.4%). This seems to be a significant measurement, of interest to oceanographers.

Caesium, of all the alkali metals, has the shortest mean residence in the ocean, about 0.9 m.y. (ref. 5). Concentrations in typical deep sea sediments, 0.9 p.p.m. , (ref. 5), imply steady transport rates that are quite consistent with mid-water concentration gradients implied by the data summarised in Fig. 1. For example, if simple constant-coefficient diffusion

Gradient of caesium in the ocean

THE major alkaline metals are extremely uniformly distributed in oceanic water relative to other solutes. Caesium occurs in the ocean in such small traces, however, that precise analysis has in the past been difficult and its distribution in the sea has remained in doubt. Samples collected at seven depths in the North Pacific in 1970 were measured using a differential flame photometer, which indicated, that caesium concentrations in the open North Pacific vary by no more than about 2% between depths of 0 and 4,000 m (ref. 1). Unfortunately, this earlier analytical procedure, and others in use, are incapable of demonstrating the still smaller anom-

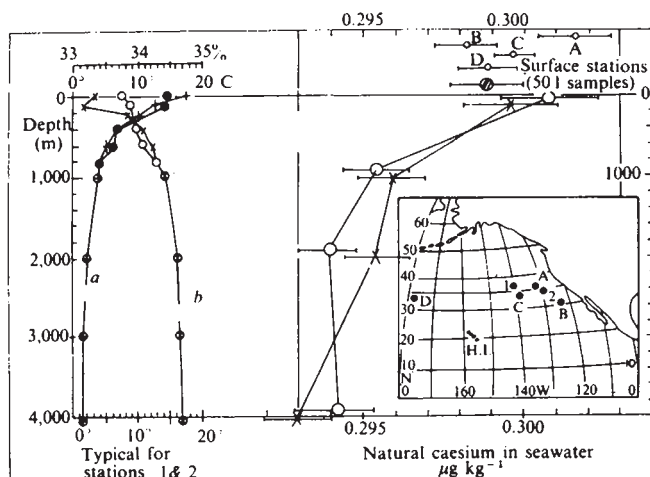


FIG. 1 Variations in natural caesium (^{133}Cs) concentrations in the North Pacific (normalised to salinity 35.00‰). The map shows positions of the stations sampled: A and B in 1970; C, D, 1, and 2 in summer 1973. Precision for caesium measurements had to be greatly advanced to make these comparisons. Tentatively a mean value of $0.299 \mu\text{g kg}^{-1}$ of Cs for North Pacific surface water was concluded. Deep water seems to have 1.4% less caesium. a, Temperature; b, salinity; open and hatched circles, deep station 1; +X, deep station 2.

through an ocean 'cap' 1,000 m thick is tentatively considered, a mean coefficient of 0.2 is required. Thinner 'caps' require correspondingly smaller diffusivities which range within values suggested by several other midwater studies⁶⁻⁷. Further assurances come from finding that the changing vertical concentration profiles⁸⁻⁹ of fallout radiocaesium, ^{137}Cs , are consistent with mixing rates approximately expressible by coefficient of these magnitudes, as are the changing concentrations of radioactive caesium observed in tissues of oceanic fish during the past decade¹⁰.

The mean surface value of $0.299 \mu\text{g kg}^{-1}$ seems free from absolute bias to well within 1%. Studies of chemical blanks and the linearity of signals with concentrations are, however, being further investigated to refine this absolute measurement. It is now evident, from comparisons of 1970 samples A and B with recent samples C, D and 1, that the large storage losses previously feared may be overcome, although to what extent is still uncertain. It is, nevertheless, evident that investigations of oceanic caesium should be referred to large contemporary samples of seawater of well established salinities, preferably deep water samples.

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Change in the current regime in the Suez Canal after construction of Aswan High Dam

MORCOS and Messieh¹ attempt to confirm conclusions by Morcos², exemplified by the statement "Comparison with older data from the Canal in September 1924, September 1933, September 1954 and September 1964 showed that a complete reversal of the current regime at that time [September 1966] had taken place."

In their recent letter¹ other observations of 1966 were presented which, they claimed, 'confirm that the southward current which has hitherto occurred in the Canal every summer, did not occur in the summer of 1966 and that the current remained northward during the whole year.'

It is difficult to see how such a statement could be maintained in face of El Sabh's^{3,4} observations of September 1966. In Morcos and Messieh's words describing El Sabh's observations "The northern part of this section, unlike Morcos's section, shows that the northern part of the canal was filled with Mediterranean water." Noting that this northern part is more than 70 km long, Morcos and Messieh's self-contradiction becomes apparent, as the Mediterranean water could not conceivably penetrate that far south, with a northgoing current prevailing all year round.

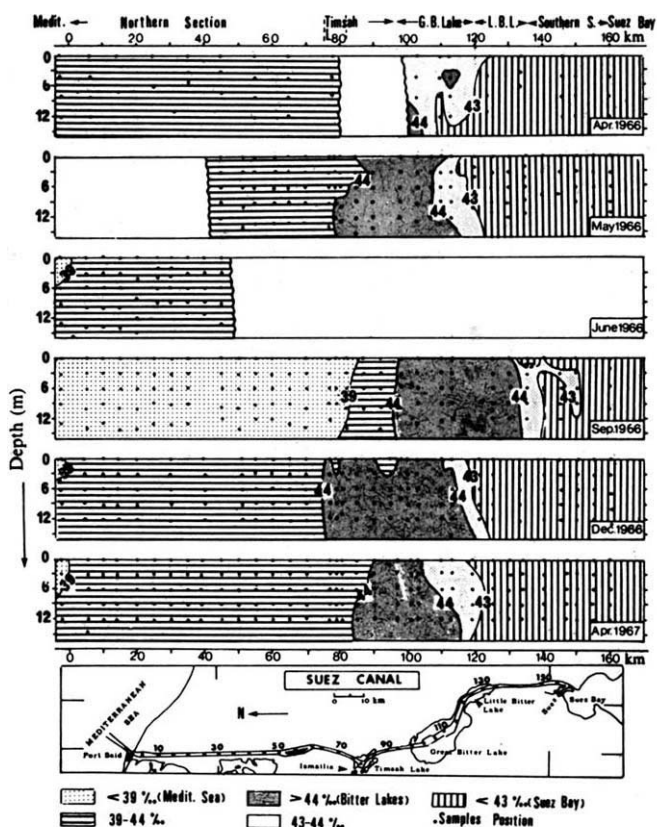


FIG. 1 Salinity with depth section of the Suez Canal in 1966, showing positions of stations and depths at which samples were taken.

The explanation is that Morcos's observations on September 29–30, 1966 were made after the normal September situation has ceased and the northgoing current resumed flowing.

As to their assertion that the net current in the southern part of the Canal was also northgoing, based on observations that the Great Bitter Lake (GBL) water did not reach the Suez Bay, this is also a conclusion not warranted by the evidence advanced.

For the GBL water to reach the Suez Bay, it has first to fill the Little Bitter Lake, then fill the southern Canal and only then can it be detected in the Suez Bay. But it can move southwards to the Little Bitter Lake, the southern part of the Canal and even into the Suez Bay. The southern limit of GBL water will depend upon the net speed and duration of the motion. Comparing the September 1966 observations with those in May and December 1966 (Fig. 1) the GBL water has moved south to fill Little Bitter Lakes in September. Because the cross-sectional area of the Little Bitter Lake is five times the mean cross section of the Canal proper, the filling of this lake (~15 km long) is equivalent to filling about 75 km of the Canal proper. It is admitted that the southern motion was not as strong in 1966 as previously observed, but it would be a mistake to insist that it did not occur.

Our conclusion is that the usual reversal of the Suez Canal regime occurred during 1966, as it did in previous years. The magnitude of the reversal in that year was not as great as previously observed, and further observations are necessary to establish the magnitude of the influence of the cessation of the Nile flood on that reversal, but certainly it did not stop it.

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water flows from the Mediterranean to the Bitter Lakes while the current in the southern canal continues to flow northward from the Red Sea to the Bitter Lakes. Examples given by Baussan were July–August 1935, June, July, August 1936, and June 1937. In these months the water flowed from both ends of the canal towards the Bitter Lakes. This is the most likely regime since, according to Miller and Munns², compensation must be made for the high evaporation from the Bitter Lakes during this season. Thus, Hassan and El Sabh's observation that Mediterranean waters penetrated 70 km into the northern canal does not necessarily imply that the current throughout the canal (the net outflow) was southwards.

My studies³ of current measurements in the promontory of Kabret, between the two basins of the Bitter Lakes, indicate that the resultant currents are much weaker and less variable in magnitude and direction, and may even show a sign opposite to that of the stronger currents in the southern canal. The water mass of the Great Bitter Lake (GBL) increases in volume and salinity in summer because of high evaporation and slow currents over the salt bed^{4,5}. This is most easily observed in transitional periods when water flows from both ends towards the Bitter Lakes as a result of the long residence time over the salt bed. The Little Bitter Lake (LBL) is an integral part of the Bitter Lakes Basin and in the absence of a strong northward current is filled by the water mass of the GBL as a result of the expansion of this water mass in volume and its increase in salinity. This is enhanced by diffusion and turbulence processes and should not be taken as evidence of a southward current in the canal, as suggested by Hassan and El Sabh. A similar distribution, showing that the LBL filled with GBL water mass, was observed in August and November 1931, July, August, October and November 1933, June and July 1935 and June 1955. These are months with transitional characteristics or even a weak northward current.

In the various parts of the Suez Canal, currents change direction over periods of hours and days as a result of tidal and non-tidal factors. The main seasonal criterion, however, is the net outflow from the canal, which quantitatively defines the current regime. This is probably an appropriate expression of the final resultant of the complex system of currents in the canal.

Continuous recording of currents at two points in the southern and northern canals has quantitatively demonstrated a net outflow into the Mediterranean Sea during most of the year and a net outflow into the Red Sea during late summer. For example, in 1935 there was a net outflow of $5,250 \times 10^6 \text{ m}^3$ into the Mediterranean during the first period, and of $150 \times 10^6 \text{ m}^3$ into the Red Sea during the second period⁶. Passing through the Great Bitter Lake, this latter outflow greatly increases the salinity in the Suez Bay during August and September every year. It has been demonstrated that such an increase in salinity did not take place at all during 1966⁷ and it can be safely stated that the net outflow from the canal into the Red Sea previously observed every late summer, did not occur in the summer of 1966.

Finally, Hassan and El Sabh admit that an unusual phenomenon occurred in the current regime in the Suez Canal during 1966 and that "further observations are necessary to establish the magnitude of the influence of the cessation of the Nile flood on the reversal of the current". In their view, this unusual phenomenon is only a change in the magnitude of the reversal, whereas it is, according to the evidence present here, a fundamental change in the seasonal pattern of the current regime. My conclusion is that the northward current regime in 1966 was followed by a transitional period with water flowing from both ends to the Bitter Lakes. By mid September the current had

DR MORCOS replies: Hassan and El Sabh compare one season with another by using their five sections of 1966. My approach is to study the seasonal cycle of 1966 in the light of the seasonal observations made during the preceding years, with a view to detecting any change that could have taken place after the construction of the Aswan High Dam.

Between 1933 and 1937, the scientists of the Suez Canal Company conducted an extensive 'quantitative' study of the water regime of the canal by registering the mean water level and currents using five tide gauges and two continuous recording current meters north and south of the Great Bitter Lake. Baussan¹ concluded that between the northward current in winter and the southward current in late summer, there is a transitional period during which the current is only reversed in the northern canal. Thus

regained its northward direction without being reversed to the south.

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Time-dependent viscosity effects measured on an impact microviscometer

THE viscosity of a fluid has been shown by conventional viscometers to rise approximately exponentially with pressure. Such viscometers always require a long period of time to build up the pressure on the fluid, typically an hour or more to reach pressures common in elastohydrodynamic contacts. Since a typical residence time for a fluid in a contact would be less than 0.01 s, Fein has suggested that fluids might not reach equilibrium viscosities instantaneously¹. Until now there has been no direct experimental evidence to support this suggestion.

We have already published some viscosity-pressure data measured on an impact microviscometer², and Hutton and Phillips have found the viscosity-pressure characteristics of one of the fluids using a Couette viscometer³. A large discrepancy is seen when the two sets of results are compared, except at the very low pressure end. Above 60 MN m⁻² the discrepancy increases steadily until, at the highest pressure reached, there is a difference of almost four decades in viscosity. We suggest here a reason for this enormous discrepancy.

The technique used to determine viscosity has been described². In brief, the elastohydrodynamic film of liquid trapped between a flat surface and a loaded ball is measured using a new interferometric technique. From the variation of film thickness with position and time the viscosity can be calculated using Reynolds equation in polar co-ordinates.

The pressure field is found from the elastic deformation of the surfaces. The shear stress τ , given by $\tau = (1/2)h\delta p/\delta r$, is quite large, reaching 50 MN m⁻². These stresses might cause the fluid to be non-Newtonian (K. L. Johnson, personal communication) thus explaining the difference between our results and those of ref. 3. In the dimple the shear stresses rose to a maximum at about $r = 0.3R$ (where R is the radius of the entrainment measured from the centre), and fell to zero at the centre where $\delta p/\delta r = 0$ and at the edge where h is small.

If the critical shear stress, above which the liquid is non-Newtonian is less than the maximum shear stress, the viscosity measurements would only be correct near the centre and edge of the entrainment, but would be too low in the intermediate position. Typical viscosity measurements with the impact microviscometer show very little increase of viscosity with pressure in the high pressure regions, the viscosity measured at $0.3R$ is very much the same as the viscosity near the centre. Taking into account a shear stress

effect lowering the effective viscosity would mean that the 'true' viscosity is considerably larger than the observed values in the intermediate region. This implies that 'true' viscosity reaches a peak as the pressure is increased and then falls at the extreme high pressures, which is most unlikely. Thus it is reasonable to conclude that the shear stresses here are not high enough to cause non-Newtonian behaviour in the fluid.

The next factor to be considered was whether there could be any time dependence of the viscosity on the pressure step imposed by the ball. In previous measurements there appeared to be no systematic variation of results taken at successive intervals of time as the fluid leaked out of the entrainment. It was realised however, that compared with the time intervals of the measurements these results were taken a long time after the pressure step was applied.

The viscosity of one fluid was therefore measured using a ciné-camera at 400 frames s⁻¹ to obtain viscosity measurements at times from 0.01 to 5 s after the imposed pressure step. Some results were also taken on a new apparatus which, by using a plate made of sapphire instead of glass, tolerates much higher pressures. Because of the higher pressure, the fluid took much longer to flow and results could be obtained at times of up to 200 s after the pressure step. The results for the chosen fluid, a paraffinic cylinder stock B P 10.65, are shown in Fig. 1. There is a definite time delay of the viscosity rise after a pressure step. At 800 MN m⁻² the viscosity varies from 3×10^4 N s m⁻² after 0.015 s, to 3×10^6 N s m⁻² after 105 s.

The polyphenyl ether tested in the earlier experiments^{2,3} was then measured at the longest possible time after the pressure step to see if there would then be a better correlation with the results of Hutton and Phillips. The new results, together with the earlier ones^{2,3} are shown in Fig. 2. The new results show a better correlation although the curves still diverge at a pressure of 150 MN m⁻². This is to be expected since the technique of Hutton and Phillips² requires a considerably longer time to reach this pressure than the 130 s in our experiments, so the discrepancy in the results can probably be attributed to time-dependence effects. Also included in Fig. 2 for comparison is a viscosity-pressure characteristic obtained for polyphenyl ether 5P4E⁴. This was derived from traction data on the fluid as it passed through a rolling/sliding elastohydrodynamic contact at 99° C and therefore refers to time scales of much less than

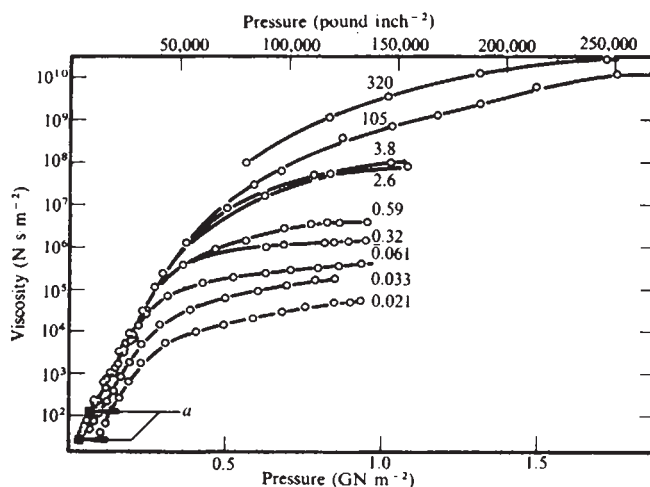


Fig. 1 Variation of viscosity with pressure of fluid BP 10.65 paraffinic cylinder stock, showing time delay of viscosity increase after a pressure step. The figures following each curve indicate the amount of time which has elapsed (s) since the first photograph (see text). a, Viscosity measured on a conventional viscometer.

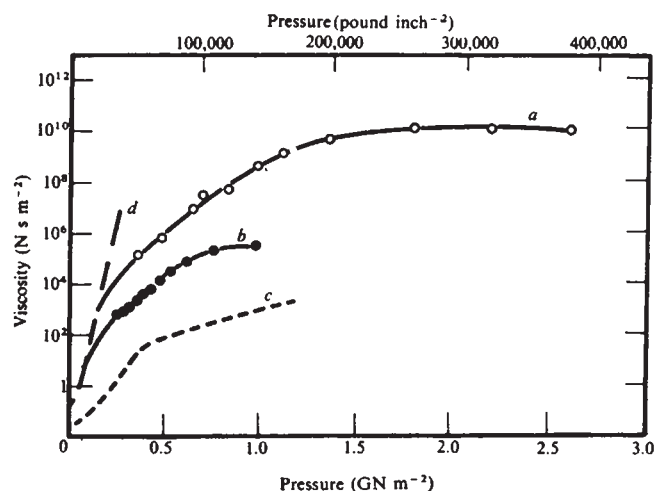


FIG. 2 A comparison between the new results and results obtained in earlier experiments. *a*, Fluid 5P4E at 23° C after 130 s; *b*, fluid 5P4E at 23° C after 5 s; *c*, fluid 5P4E at 99° C, derived from traction data⁴; *d*, fluid 5P4E measured on a Couette viscometer³.

our results in Fig. 2. No direct comparison can, therefore, be made but it is interesting to note that the same general behaviour is found.

In conclusion, it seems that time-dependent variation of viscosity following a pressure step, first proposed by Fein¹, has been experimentally established. This will have a profound effect on estimates of viscosity inside elastohydrodynamic contacts. We are now investigating the time dependence for other fluids.

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Surface structures of gibbsite goethite and phosphated goethite

INFRARED studies of surface hydroxyls on solids of high area have been limited to almost anhydrous materials prepared at elevated temperatures. These studies have made an important contribution to the understanding of surface catalysis, but have little relevance to adsorption processes that occur on moist surfaces at ambient temperatures. Such processes contribute to a wide range of everyday phenomena, including the retention of fertilisers and pesticides in soils.

Here we establish that the surfaces of the crystalline hydroxides gibbsite (γ -Al(OH)₃) and goethite (α -FeOOH), both common soil components, are accessible to infrared study, and that their surface structures are well defined and closely related to their bulk structures. The observation of

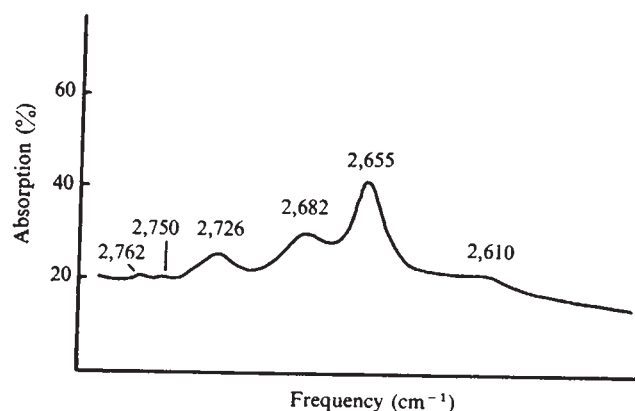


FIG. 1 Infrared absorption of surface Al-OD groups on the (001) face of gibbsite in the form of a polycrystalline film (6.2 mg cm⁻²). Weak bands at 2,762 and 2,750 cm⁻¹ may arise from Si-OD of adsorbed silicic acid; the band at 2,610 cm⁻¹ is due to bulk OD.

these surface hydroxyls opens the way to the study of their involvement in surface reactions.

Electron microscope analysis shows the synthetic gibbsite¹ was in the form of thin hexagonal plates of diameter about 250 nm and thickness 9 nm. Their surface consisted principally of (001) faces with a calculated area of 96 m² g⁻¹, whereas edge faces represented only 8 m² g⁻¹. On evaporation from suspension, this material formed excellent self-supporting films with the crystal plates parallel to the film plane.

The infrared spectrum of this gibbsite is dominated in the 3,000 to 4,000 cm⁻¹ region by absorption due to the stretching vibrations of bulk hydroxyl groups, which give bands at 3,622 cm⁻¹ and 3,529 cm⁻¹ polarised in the (001) plane, and a third at 3,460 cm⁻¹ polarised perpendicularly to that plane. Gibbsite consists of infinite planes of close-packed hydroxyl groups parallel to (001), the planes being bound together in pairs by aluminium ions to form a layer structure. Thus the perpendicular 3,460 cm⁻¹ band can be correlated with hydrogen bonds between layers (2.82 to 2.87 Å long²) and the 3,622 cm⁻¹ and 3,529 cm⁻¹ bands correspond to longer hydrogen bonds between hydroxyls within the same plane (3.29 Å and 3.13 Å long according to ref. 2; a longer in-plane OH-OH separation of about 3.38 Å probably involves no interaction).

Absorption by these bulk hydroxyls largely obscures the absorption bands of surface Al-OH groups, but a brief (10 s) treatment of a gibbsite film with D₂O followed by evacuation revealed surface Al-OD groups absorbing at 2,726 cm⁻¹, 2,682 cm⁻¹ and 2,655 cm⁻¹ (Fig. 1), which were lost again immediately on exposing the films to water vapour. The corresponding frequencies of surface Al-OH groups would be 3,690 cm⁻¹, 3,629 cm⁻¹ and 3,588 cm⁻¹ but only the first can be seen in evacuated films because of the strong absorption by bulk hydroxyl groups. All three surface absorption bands are polarized in the plane of the layers, so the OH groups that were involved in interlayer bonding within the crystal (absorbing at 3,460 cm⁻¹) must link OH groups within the surface plane, and probably absorb at 3,690 cm⁻¹. This weak interaction leads to a lengthening of other in-plane OH-OH separations, so that hydroxyls that absorb at 3,529 cm⁻¹ and 3,622 cm⁻¹ within the crystal absorb at 3,588 cm⁻¹ and 3,629 cm⁻¹ on the surface.

The synthetic goethite used was prepared as was specimen 1(c) of ref. 3, and, like that specimen, consisted of thin laths with the (100) faces predominant, typically measuring 150 nm in the [001] direction, 30 nm wide and 10 nm thick. The calculated area of the (100) faces is 45 m² g⁻¹, with edge planes contributing about 15 m² g⁻¹.

The goethite structure (Fig. 2) can be regarded as being built from strips of condensed Fe(O, OH) octahedra, each

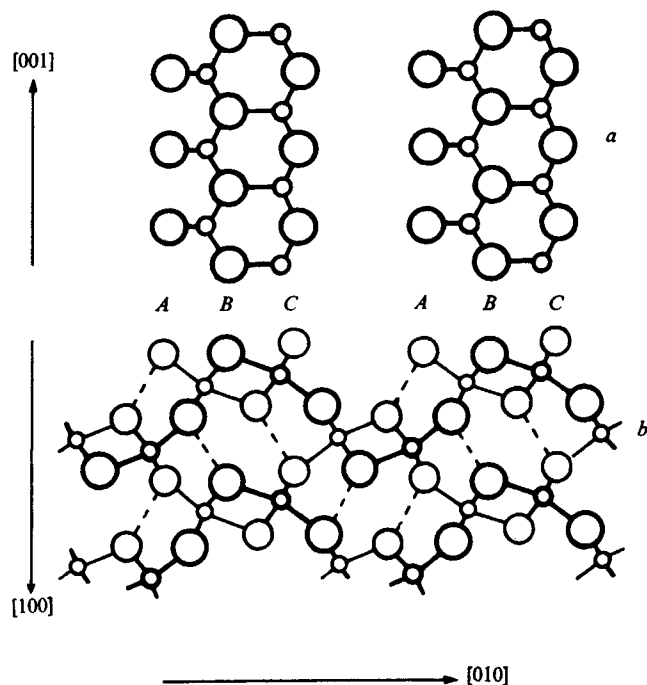


FIG. 2 Plan (a) and section (b) of the (100) face of goethite (after Bragg and Claringbull⁵).

strip being two octahedra wide. The strips, which are seen in plan in Fig. 2a, and in section in Fig. 2b, share O^{2-} ions along their edges to give an open structure in which the gaps between the strips are bridged by hydrogen bonds (dashes in Fig. 2b) involving OH groups shared between three Fe^{3+} ions. These bulk hydroxyls absorb at $3,145\text{ cm}^{-1}$. The same hydroxyls (labelled B in Fig. 2) are also exposed on the (100) surface together with two new types of hydroxyls (labelled A and C) which arise from protonation of the oxide ions along the edges of the strips. Of these surface hydroxyls,

types B and C cannot form hydrogen bonds within the surface, since all adjacent hydroxyls are coordinated to a common Fe^{3+} ion, but type A hydroxyls, each coordinated to only one Fe^{3+} , can form bonds $3\text{ \AA}$ long with each other.

In the spectra of films of goethite examined *in vacuo* (Fig. 3a) type A hydroxyls absorb at $3,486\text{ cm}^{-1}$ and the non-bonded hydroxyls absorb at $3,660\text{ cm}^{-1}$. These surface hydroxyls are readily converted to OD, absorbing at $2,700\text{ cm}^{-1}$ and $2,581\text{ cm}^{-1}$, by brief (1 min) treatment with D_2O , and they interact with adsorbed water when the films are exposed to air, as shown by broadening and displacement of the absorption bands.

Goethite that had been treated with $200\text{ }\mu\text{mol per g H}_3\text{PO}_4$ or NaH_2PO_4 before preparing films showed no absorption due to type A hydroxyls (Fig. 3b), thus confirming the hypothesis of Atkinson *et al.*⁴ that phosphate replaces these hydroxyls, forming bridges between adjacent Fe^{3+} . The spectra also demonstrate an interaction between phosphate and B or C type hydroxyls, resulting in new bands appearing at $3,671\text{ cm}^{-1}$ and $3,646\text{ cm}^{-1}$. This could represent the effect of a hydrogen bond between surface $(FeO)_2PO\cdot OH$ groups, acting as proton donors, and type C hydroxyls. These and other reactions of surface hydroxyl groups on hydroxides are being actively investigated.

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Gravitational charge, hadron hydrodynamics and Gödelised hadrons

I DISCUSS here the consistency of approach which is not immediately apparent when current approaches to particle physics are compared. The parton model, *f* gravity and the hydrodynamical model of multiple particle production in high energy hadron collisions complement each other in remarkable ways and point to a useful general formalism for particle physics problems in such diverse applications as mass spectra through collisions to the first picoseconds of the Universe according to the big-bang theory.

Motz has obtained an expression for gravitational "charge" in the interior of fundamental particles based on the Weyl theory of gauge invariance¹ and on the Schwinger (actually Bohr-Sommerfeld) quantisation procedure² which implies a value for the gravitational constant of $\sim 1.8 \times 10^{31}$ (c.g.s. units) within a proton (or any hadron). Bakesigaki and Inomata³ have derived a value of $G \sim 1.2 \times 10^{32}$ from the strong *f*-gravity theory⁴ and a model of hadrons as de Sitter spacetimes possessing rotational degrees of freedom (relativistic "rotators")⁵ by obtaining a mass formula for hadrons

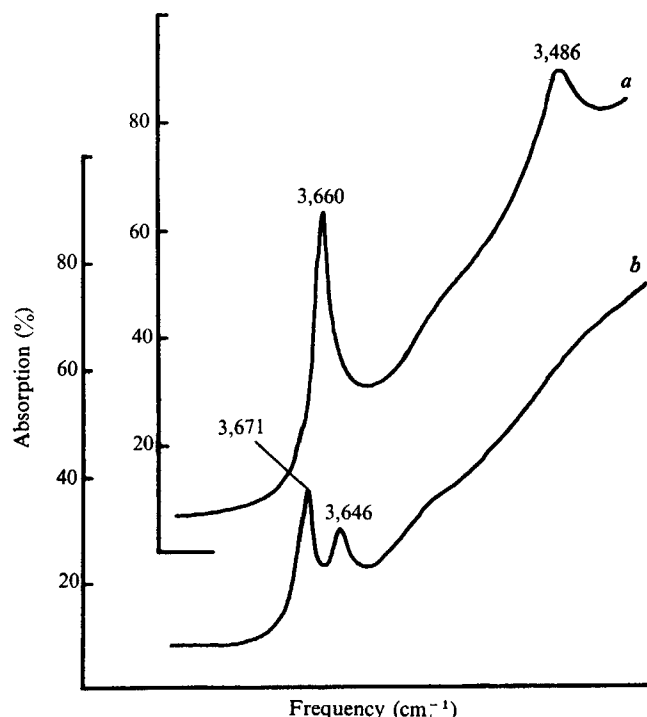


FIG. 3 Infrared absorption of surface OH on: a, Goethite; b, phosphated goethite. Both in the form of polycrystalline deposits (5.4 mg cm^{-2}).

under the assumption of conformal $SO(4, 2)$ symmetry (invariance) being broken into the $SO(4, 1)$ symmetry group, the masses then corresponding to the energy levels specified by the angular momentum quantum number J .

What other sort of relativistic rotator is consistent with the existence of such large values of the gravitational constant under a gravitational charge hypothesis applied to hadronic matter (strongly interacting fundamental particles)? The simplest models should always be considered first, so I will look for classical phenomena which may still be useful for qualitative description in the quantum domain of the hadrons. The Landau hydrodynamical-thermodynamical fluid description of multiparticle production^{6,7} has recently been reconsidered with some success^{8,9}. So it seems useful to seek a fluid description, and just look at the proton as an infinitely bootstrapped¹⁰ hadron ground state. Another semi-classical approach has involved the use of the cosmological constant in the Einstein equations as the inverse square Compton wavelength of the f^{2+} -meson and interpreting the meson spectrum as that of an oscillator, thereby coming within half a pion mass of the proton's mass for the ground state of a hadron¹¹. I note also that the parton model of hadrons containing an almost infinite number of almost light-like particles¹² lends itself easily to a statistical-fluid description of the hadron in terms of its constituents. So I conclude that it is useful to examine a fluid description of the hadrons in which the cosmological constant also may have meaning.

One possibility is Gödel's universe¹³. To examine this I work with Gödel's spacetime line element in the form $ds^2 = (dx_0 + \exp(ax_1) dx_2)^2 - dx_1^2 - (1/2) \exp(2ax_1) dx_2^2 - dx_3^2$, where a is related to the rotation velocity w and the speed of light, c by $w = (ac/2)^{1/2}$ (ref. 14). The Einstein field equations for such a line element then imply that the density u and pressure p of the matter are related by $p = u = a^2$ (in geometrised units), implying unusual properties for such matter¹⁵. An alternative interpretation of the Gödel solution would constrain the fluid to $p = 0$, $u = -\Lambda/4\pi$, where Λ is the cosmological constant, and $\Lambda = -a^2/2$ (ref. 14).

So there are two possible approaches. In the first case, one fixes the field of the proton near its surface from application of the Kerr-Newman metric in an f -gravity form¹⁶, thereby fixing the curvature for the density u and then matching that curvature *a priori* to $u = a^2$ in the Gödel solution. The second case would involve self-consistent use of the relation $u = a^2$ entirely within the Gödel solution but that would make no reference to f gravity and allow no connection of possible exterior and interior solutions to the field equations $G_{ab} = -k T_{ab}$ for a fluid hadron, which is a part of the motivation for this work. I use the former.

Let the rotation w be given by the usual relation of angular momentum L to moment of inertia I ($w = L/I$) and let $I = Q_g MR^2$ where M is the particle mass, R is its radius and Q_g is the gravitational charge. Inserting the parameters for the proton of $M = 1.6 \times 10^{-24} g_m$, $L = \hbar/2$ ($\hbar = \text{Dirac's constant}$), and $R \sim 10^{-13}$ cm, using the Kerr-Newman curvature from the components of the Riemann tensor in the equatorial plane ($R_{\theta\phi\theta\phi} \sim GM/c^2 r^3$), $u \sim 1.1 \times 10^{-12}$ cm², and further augmenting the gravitational effect of the mass implied by the ultrarelativistic 'equation of state' $p = u$ (that is, doubling the mass), we find $Q_g \sim 3 \times 10^{17}$. Applying the charge relation of Motz² ($Q = G^{1/2}$) we find $G = Q_g^2 \sim 9 \times 10^{34}$. This is a larger value of G than found by others^{1,2} but it does augment their impression of extremely large values of G within hadrons.

The values may be brought into agreement by detailed consideration of the charge distribution in the particle¹⁷ or its mass distribution (these distributions being possibly of equal value here because an application of the parton model with a vector gluon interaction between partons has shown

that the two distributions are related and may be identical¹⁸), by perhaps allowing a density distribution $u \sim u_0 \exp(-x_l/c)/x_l$, where l is a scale length. That is not unreasonable since it is the coupling of the field and its source which produces gravitation and this source distribution has the structure of the Yukawa field. Peak's consideration of the relation of strong gravity and the Yukawa potential, indeed, derives an equation which implies such a form of u (ref. 19). Such a distribution yields a decreased requirement on Q_g in the moment of inertia, implying a value of $G \sim 3 \times 10^{32}$. I have found that the Einstein tensor for the inhomogeneous Gödel model calculated from the distribution $u_0 \exp(-x_l/c)/x_l$, with $a = a(x_l)$, contains non-zero G_{13} terms corresponding to stresses in the matter. These determine Maclaurin spheroids which may be matched to the quadrupole moment and angular momentum relation in the Kerr-Newman solution and its axis-symmetric null surfaces.

Using the cosmological constant instead gives the same results. The only problem, that of consistency with present work in particle physics, involves the $p = 0$ equation, but hadron models with such a constraint are under construction by a group at Massachusetts Institute of Technology and it has been shown that a hadron gas under conditions of compaction of the early Universe similar to that of the maximum temperature ($T_m = 160$ MeV) hydrodynamical model²⁰ may obey an equation of state such that u is so much greater than p that p may be set equal to zero by comparison with u (ref. 21).

The above consideration is worth noting because there now exists the formalism of the tilted universe in cosmology²² which is immediately adaptable to the $f - g$ gravity theory by adding the coupling terms in the field equations to the cosmological equations⁴, with or without cosmological constant (with or without altered constraints on the equation of state such as $p = 0$). But the tilted frames are specified by the same coordinate transformations which specify the infinite momentum frames of the parton model¹²; so either way, a complete formalism now exists to do the complete physics of the first picoseconds of the big-bang origin of the universe, including gravitation, hydrodynamics and thermodynamics, and particle physics under the constraints of completely and consistently specified models.

There is, in conclusion, a remarkable consistency in these new gravitationally/hydrodynamically oriented approaches to the structure of the fundamental particles and a much greater range of applicability (from hadrons to 3C295) than has been attempted.

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BIOLOGICAL SCIENCES

Specific recognition of the isolated R17 replicase initiator region by R17 coat protein

Coat proteins from the RNA bacteriophages of groups I (R17, MS2, f2) and III (Q β) specifically bind to their homologous RNAs and serve as translational repressors of replicase synthesis¹. In the case of R17, a fragment of the genome which is protected by coat protein from T₁ RNase digestion² contains not only the ribosome binding site of the replicase cistron³, but also the preceding intercistronic region and 23 nucleotides (including the terminator triplets) from the coat gene⁴. Physical studies described in the previous papers^{5,6} suggest that the structure of this isolated R17 fragment consists of two hairpin loops joined by a single-stranded region; the coat protein binds in such a way as to alter the behaviour of only one of these helical regions⁵, that which contains the initiation site for the replicase gene. Here I show that this portion of the fragment alone is sufficient for recognition by the coat protein.

Isolated protein synthesis initiator regions from R17 RNA⁷ provide a convenient source of the 3' terminal one-third of the coat protein-protected fragment (helix *b* of Fig. 1, ref. 5). Such ribosome binding site preparations contain a mixture of the beginnings of the three R17 genes, each present in several size variants as illustrated in Fig. 1.

Table 1 and Fig. 2 show that the replicase initiator region can be both selectively and efficiently bound to Millipore filters by R17 coat protein. If the mixture of coat protein

and ribosome binding sites is treated with T₁ RNase before filtration, only those oligonucleotides which are at least partially contained in helix *b* (Fig. 1, ref. 5) are protected. Interestingly, Q β coat protein shows some affinity for the R17 replicase initiator region suggesting that it recognises a structurally similar site in its own RNA; the fact that the binding is less efficient and abolished by T₁ RNase probably explains why no protection of whole R17 RNA by Q β coat protein was previously observed².

The capacity of R17 coat protein to recognise the replicase

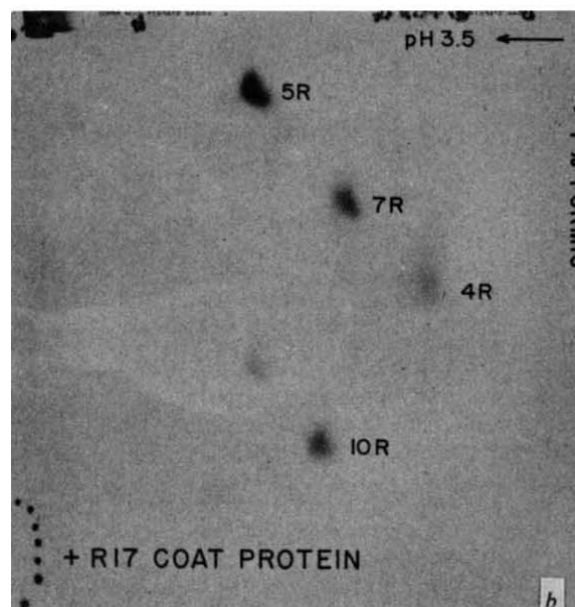
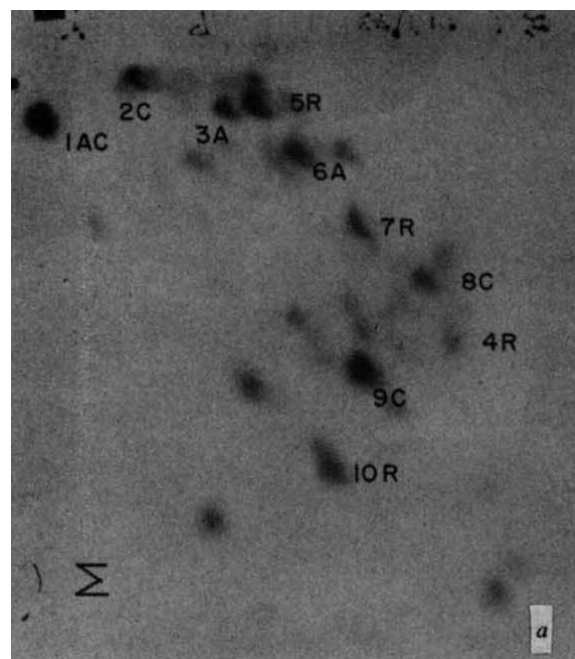


Fig. 2 T₁ RNase fingerprints of R17 initiator fragments (a) before incubation with, and (b) trapped on Millipore filter by R17 coat protein. Data is from experiment 1 of Table 1. Spots were identified by subsequent analysis as: 5R-AUUACCCAUG, 7R-AAACAUG, 4R-ACAACAAAG and 10R-UCG from the replicase site; 6A-ACCUAUG, 3A-AUCCUAG, and 1AC-UUUG from the A site; 9C-CAUG, 2C-CUUCUACUUU, and 1AC-UUUG from the coat protein initiator region.

A protein

AU · UC · U AG · GAG · GUU · UGA · CCU · AUG · CGA · GCU · UUU · AGU
 Coat

AGAG(c)C · UCA · ACC · GGG · CUU · UGA · AGC · AUG · GCU · UCU · AAC · UUU

Replicase

AA · ACA · UGA · GGA · UUA · CCC · AUG · UCG · AAG · ACA · ACA · AAG

Fig. 1 Oligonucleotide sequences of the isolated R17 initiator fragments⁴. Bold type indicates that portion present in the major size variant from each site as judged by homochromatography³ and by the relative yields of component T₁ oligonucleotides (see Fig. 2a).

TABLE 1 Binding of initiator fragments by coat protein

	Aliquot of initiator fragment preparation	c.p.m. per $^{32}\text{PO}_4$ +R17 coat	+Q β coat	+R17 coat +T $_1$ RNase
Experiment 1				
5R	112	152	67	
7R	59	97	45	
4R		44	15	
6A	136	11	27	
3A	57	4	15	
9C	274	13	27	
2C	73	1.6	9	
1AC	378	17	53	
Ratio of sites (replicase: A: coat)	1: 1.2: 2.5	1: 0.07: 0.09	1: 0.40: 0.40	
% Replicase site bound		30	11	
Experiment 2				
5R	207	59	18	20
7R	145	51	6	18
4R		17	6	—
10R		53	20	5
6A	215	—	6	—
3A	121	—	—	—
9C	563	—	9	—
2C	164	—	3	—
1AC		—	—	—
Ratio of sites (replicase: A: coat)	1: 1: 2.7	1: < 0.05: < 0.08	1: 0.3: 0.5	
% Replicase site bound		24	7	

R17 protein synthesis initiator regions were prepared by ribosome protection, isolated and characterised as described in ref. 7 and Fig. 2a. Reaction mixtures contained in 60 μl of TMK buffer (0.1 M Tris-HCl, pH 7.5; 0.01 M Mg acetate; 0.08 M KCl); 32 μg of isolated R17 ribosome binding sites (of which only 1–2% are the radioactive sites, the remainder being rRNA and tRNA fragments⁷) and 7.6 μg freshly prepared R17 or Q β coat protein⁸, or bovine serum albumin (BSA). After 10 min at 0° C, T $_1$ RNase at 0.1 mg ml⁻¹ was added as indicated and incubation continued for 30 min at 25° C. Samples were filtered through HA Millipore filters (24 mm) and the RNA extracted as described in ref. 2. Final preparations were analysed by two-dimensional T $_1$ fingerprints (Fig. 2b) and the above oligonucleotides counted and analysed. In both experiments, controls with BSA rather than coat protein, gave no detectible spots on the fingerprints. The oligonucleotides are identified in Fig. 2. Ratios of the sites were calculated from the relative yields of the underlined oligonucleotides, which contain the three initiator AUGs. The % replicase site bound was calculated from the fraction of replicase sites in the counts originally trapped on the filter. Spots containing less than 20 c.p.m. total were not detected and are indicated by —; spots were not counted where no value appears.

TABLE 2 Rebinding of coat protein-protected fragments

	Addition at		Filter at
	0 min	10 min	20 min
59-nucleotide fragment total, 22,650	Coat	—	5,800
	Coat	T $_1$ RNase	5,100
	T $_1$ RNase	Coat	108
	—	—	47
29-nucleotide fragment total, 10,680	Coat	—	2802
	Coat	T $_1$ RNase	2616
	T $_1$ RNase	Coat	60
	—	—	25

The 59- and 29-nucleotide fragments were prepared as described in ref. 5; after elution from the gel they were characterized by T $_1$ fingerprint analysis to be approximately 90% pure. Rebinding reactions were performed in 50 μl of TMK buffer at 25° C containing (as indicated): 3.8 μg freshly prepared R17 coat protein⁸, 1.4 μg of the 59-nucleotide or 0.7 μg of the 29-nucleotide fragment (a 4:1 molar ratio of coat protein to RNA), and 0.05 mg ml⁻¹ T $_1$ RNase. After dilution with 1 ml of TMK buffer, the samples were filtered with two 1 ml rinses through HA Millipore filters and counted in toluene scintillation fluid.

initiator region, as opposed to the 59-nucleotide RNA segment studied in refs 5 and 6, can also be examined directly since a T $_2$ digest of the coat protein R17-RNA complex yields both fragments (see Fig. 1, ref. 5). Table 2 shows that the 29- and 59-nucleotide fragments are retained on a Millipore filter by coat protein with comparable efficiency.

Thus it may be concluded that the 23 nucleotides at the

3' terminus of the 59-nucleotide fragment isolated from the R17 RNA-coat protein complex are sufficient for recognition by the protein. As originally suggested by Bernardi and Spahr², helix *a* (the coat cistron terminator region) most likely remains attached because of its great stability and resistance to RNase.

Must the R17 replicase initiator region assume a helical form to be recognised by coat protein? Its component T $_1$ oligonucleotides, which contain the sequence from the turn of hairpin *b* intact in oligonucleotide 5R (Fig. 1), are not efficiently rebound (Table 2); and studies presented in accompanying papers^{5,6} indicate that helix is formed by this portion of the 59-nucleotide fragment under physiological conditions. Formation of a secondary structure which sequesters the initiator AUG could explain the inability of *E. coli* ribosomes to recognise the isolated replicase initiation site efficiently⁷. In the intact R17 RNA, on the other hand, the region may well assume one of several alternative configurations: an extended form, in which the initiator AUG may become available for ribosome binding after translation of an early portion of the coat gene; or the helix, which is subject to coat protein recognition and translational repression.

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High resolution proton NMR study of an isolated fragment of R17 bacteriophage mRNA

HIGH resolution nuclear magnetic resonance (NMR) spectra of the hydrogen-bonded protons in helical regions of nucleic acids are an informative monitor of the base pairing¹. Studies of about 10 purified tRNAs and of model systems have shown that the ring NH protons of uracil and guanine, when involved in Watson-Crick base pairing, give resonances between -15 and -11 p.p.m. downfield from DSS^{1,2}. Since there is only one such proton in each base pair, the intensity in this spectral region is a direct measure of the number of base pairs. Simple rules have been derived to predict the NMR spectra originating from these protons on the basis of ring currents from the adjacent bases and for tRNA the comparison between predicted and observed spectra has strongly supported the clover leaf model of base pairing. In addition, ring current shifts from bases adjacent to the helical region provided information about the structure at the end of the helix. Here we apply this approach to the secondary structure of the R17 fragment discussed in the preceding paper³. We prepared 0.5 mg of the R17 fragment according to a somewhat modified method of Bernardi and Spahr⁴, and measured the NMR spectrum of a 25 μ l sample.

Figure 1 shows the 300 MHz high resolution proton NMR spectra of the fragment at 26° C and 40° C, obtained with a Varian HR300 spectrometer after accumulation for ~7 and ~13 h, respectively. These spectra, with resonances between -15 and -11 p.p.m., are similar to those obtained^{1,2} from other nucleic acid molecules of comparable size (for example, tRNAs) although these lines are broader. Assuming that the peak at either -11.6 p.p.m. or at -14.3 p.p.m. represents one proton, the spectrum at 26° C has a total intensity corresponding to ~16 protons, in agreement with the number of base pairs expected from the structure shown in Fig. 1 of the previous paper³. Note that GU does not, in our experience, contribute a resonance in the region between -15 and -11 p.p.m.⁵

Upon raising the temperature from 26° C to 40° C, the overall form of the spectrum changes drastically. There is an appreciable intensity reduction below -13.0 p.p.m. and the line at -11.6 p.p.m. has disappeared. After maintaining the sample for many hours at 40° C the resonances subsequently obtained at 26° C were broadened, presumably due to aggregation of the sample at this high concentration. So we could not make a direct intensity comparison between the 26° C and 40° C spectra. At 40° C the intensity in the low field part of the spectrum relative to the high field part is much lower than in the 26° C spectrum. On the basis of internally consistent intensities in the 40° C spectrum its integrated intensity corresponds to ~eight protons. Loss of about eight resonances is consistent, within experimental error, with the melting of either the *a* or the *b* region of

the structure proposed for the mRNA fragment (Fig. 1 of ref. 3). To interpret the results in more detail the spectra expected for the two proposed helices were calculated on the basis of the ring current shift contributions from nearest neighbours with the results given by the stick diagram at the bottom of Fig. 1.

The melting observed by NMR can be ascribed to a certain helical region only if at least one resonance can be assigned to the hydrogen-bonded NH proton of a particular base pair in that helical region. Since the accuracy of the calculation is no better than ~0.3 p.p.m.^{1,2} it is necessary to have a resonance which is separated by at least this distance from the rest of the spectrum before it can be unambiguously identified merely by comparing the calculated spectrum with the observed. Fortunately, such a line does appear near -11.6 p.p.m., corresponding to the GC43 (GC base pair involving nucleotide number 43 from the 5' end) and this is the only resonance calculated to be at fields higher than ~12.0 p.p.m. This assignment is independent of whether we assume that A44 is looped out so that GC45 stacks on GC43 giving a calculated position of -11.8 p.p.m. or that A44 is stacked between pairs 43 and 45 giving -11.4 p.p.m. for GC43. Although we cannot distinguish between these two possibilities now, the observed position of the GC43 resonance requires that one of these two conformations exists. The disappearance of the resonance at -11.6 p.p.m. in going from 26° C to 40° C suggests that resonances of the *b* helix are broadening in this temperature range. This conclusion is corroborated by the simultaneous intensity loss in the rest of the spectrum which coincides rather well with the reso-

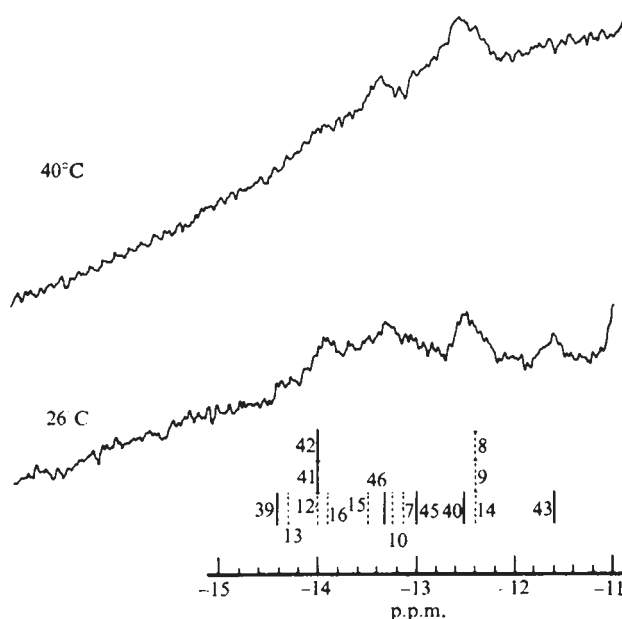


FIG. 1 The 300 MHz NMR spectra of the ring NH hydrogen-bonded protons of the R17 fragment at 26° C and 40° C. Positions are given in parts per million (p.p.m.) downfield from a DSS (2,2-dimethyl-2-silapentane-5-sulphonate) standard. The stick diagram at the bottom of the figure depicts the spectrum calculated for the secondary structure given in Fig. 1 of the previous paper³. The dashed lines correspond to the *a* helix which contains the termination codon for the coat protein gene, the solid lines to the *b* helix which contains the initiator codon for the replicase gene. Concentrations in the solution were: RNA fragment ~0.5 mM; NaCl, 50 mM; MgCl₂, 8 mM; ammonium cacodylate, 10 mM. The total sample volume was 0.025 ml in an approximately spherical NMR microcell. The R17 RNA fragment was prepared as described in Fig. 1, ref. 3. Examination of aliquots of the sample by polyacrylamide gel electrophoresis and by T₁ and pancreatic RNase fingerprinting showed it to be more than 95% pure.

nances expected from the other base pairs in the *b* helix. In particular, the loss of intensity observed around -13.1 p.p.m. is understandable if we include base pairs GC45 and GC46 in the *b* helix; without these two pairs no intensity decrease would be expected in this spectral region from the other five base pairs of helix *b*.

The NMR results on the R17 RNA fragment presented here support the interpretation of the melting behaviour given in the preceding paper³ in that the *b* helix is the first to melt, and in this way support the rules for thermodynamic stability developed to predict the secondary structure of RNAs⁷.

The results indicate that the AUG initiation triplet of the R17 replicase gene is buried in a base-paired region in this particular fragment. The combination of relaxation kinetics and NMR shows that while the fragment is predominantly base paired under physiological conditions, there is still a finite possibility of finding the helix open. Raising the temperature above 40°C would increase the fraction of time that helix *b* is open and in this way it might be possible to observe changes in the biological function of this fragment, that is initiation and translation, which could be correlated with changes in the helix-to-coil equilibrium of the helix containing the AUG initiator codon.

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(from 53S for component I to 16–18S for component II) seen during centrifugation in alkaline gradients. Such analysis shows the major effect of camptothecin treatment to be the introduction of one single-strand break or alkali-labile region per component I molecule affected.

To study the effect of camptothecin on SV40 DNA, labelled viral DNA from infected drug-treated cells was analysed by

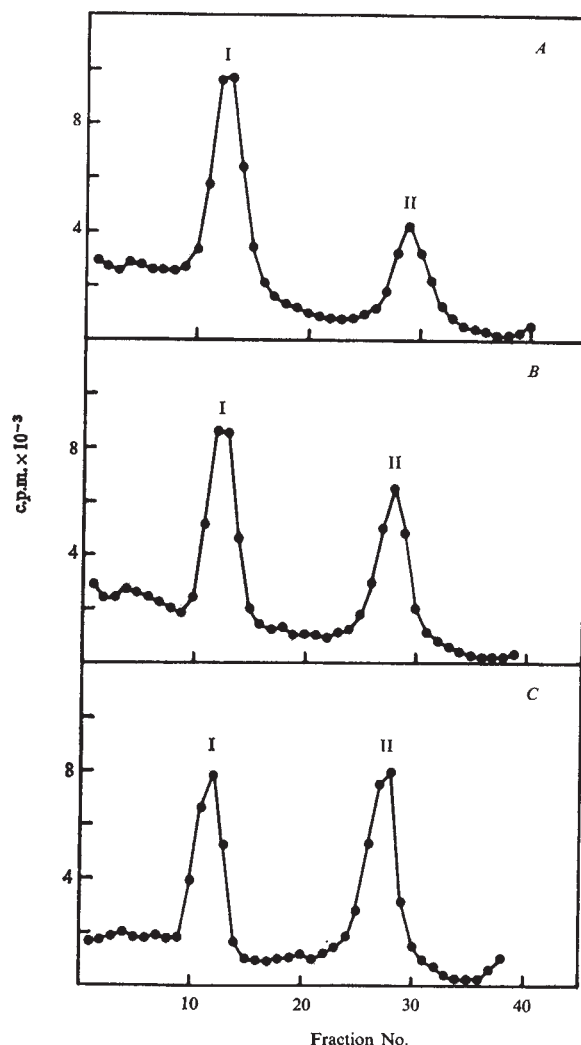


Fig. 1 Velocity sedimentation in alkaline CsCl of viral DNA extracted from cells treated with camptothecin. Confluent monolayers of Vero cells, grown in 60 mm Petri plates in Dulbecco-Vogt medium with 10% Colorado calf serum, were infected with SV40 (small plaque strain) at a multiplicity of approximately 2 plaque-forming units (PFU) per cell. The virus was allowed to adsorb to the cells for 3 h at 37°C , at which time the inoculum was replaced with medium and the incubation continued at 37°C . Fresh medium containing $20\ \mu\text{Ci ml}^{-1}$ ^3H -thymidine (Mallinckrodt, $14.6\ \text{Ci mM}^{-1}$) was added at 45 h after infection, and the incubation was continued 3 h, at which time viral DNA from one plate (A) was immediately extracted by the method of Hirt¹². Other plates were treated for 30 min at 37°C with $10\ \mu\text{g ml}^{-1}$ camptothecin (B) or $100\ \mu\text{g ml}^{-1}$ camptothecin (C) in fresh medium, followed by Hirt extraction using lysing buffer containing $5\ \mu\text{g ml}^{-1}$ camptothecin. Aliquots (0.1 ml) of the Hirt supernatants were layered on 3 ml CsCl ($\rho = 1.52$) containing $0.1\ \text{M K}_2\text{HPO}_4$ (pH 12.9), and centrifuged at 15°C for 80 min in an SW 50.1 rotor at 44,000 r.p.m. The tubes were punctured and fractions were collected as described¹⁴ on 22.8 cm diameter, segmented Whatman GF/A filter discs which were dried, then washed in a Büchner funnel with 6% trichloroacetic acid and absolute ethanol. Segments containing individual fractions were then pulled off with forceps, dried in scintillation vials, and counted in toluene-Liquiflour scintillation fluid. Sedimentation is from right to left.

Effect of camptothecin on simian virus 40 DNA

THE alkaloid camptothecin has been shown to be a potent, rapidly acting inhibitor of RNA and DNA synthesis in eukaryotic cells^{1–5}. The drug is selectively active against high molecular weight nuclear RNA synthesis^{2–5}, having no effect on mitochondrial⁶ or 4–5S RNA synthesis^{2,5}. Its effect on RNA synthesis is rapidly reversible^{1–3,5}. In addition, it has been shown that camptothecin induces alkali-labile links in DNA^{1,7–9} which are rapidly reversed by removal of the drug⁹. It was suggested that this action on DNA is responsible for the effect of the drug on RNA synthesis⁵.

Here we describe the effect of camptothecin on simian virus 40 (SV40) DNA. These molecules normally exist as either covalently closed superhelical double-stranded circles (component I) or relaxed circles containing single-strand scissions (component II). Introduction of even one single-strand break in component I molecules can be easily assayed because of the drastic change in sedimentation coefficient

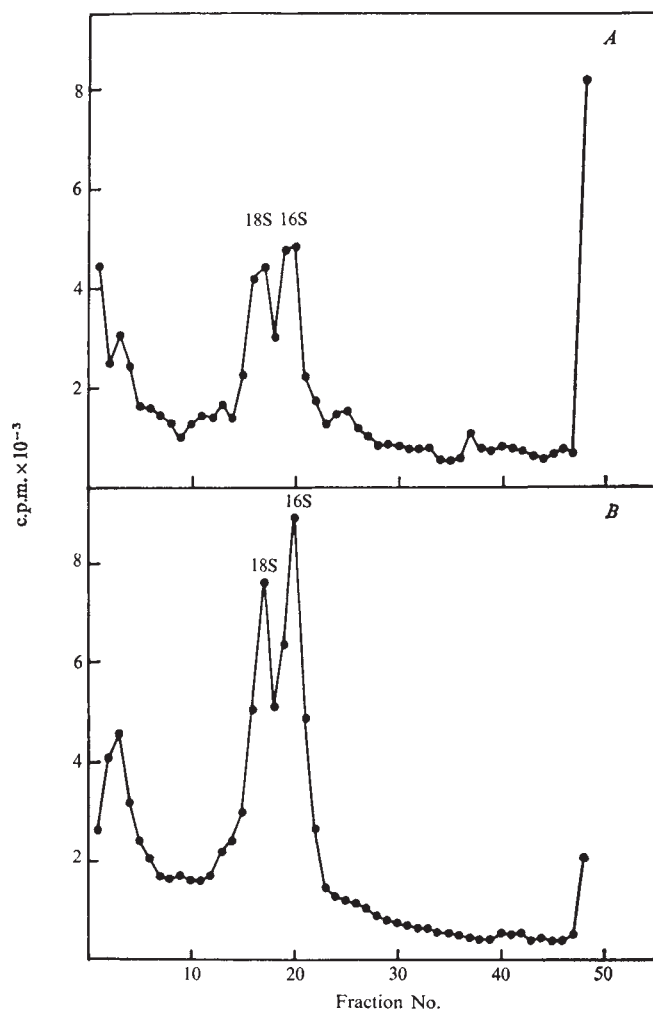


Fig. 2 Velocity sedimentation in alkaline sucrose of viral DNA extracted from camptothecin-treated cells. Aliquots (0.2 ml) of Hirt supernatants of the untreated cells (A) or the cells treated for 30 min with $100 \mu\text{g ml}^{-1}$ camptothecin (B) (see legend to Fig. 1) were layered on 11 ml 10–30% sucrose gradients containing 0.3 M NaOH, 0.7 M NaCl, 0.001 M EDTA. Centrifugation was for 16.5 h at 10°C in an SW41 rotor at 40,000 r.p.m. Fractions were collected on GF/B filters (see legend to Fig. 1). Sedimentation is from right to left.

alkaline caesium chloride centrifugation. As shown in Fig. 1A, viral DNA sediments as two bands in such gradients, component I at 53S and component II at 16–18S. Component I comprises the majority of the viral DNA molecules in normal conditions, with component II arising mainly from the random introduction of one single-strand break per component I molecule¹⁰.

When infected cells making viral DNA are treated with camptothecin for a short period of time, some of the component I molecules are converted to component II. Treatment of cells containing labelled viral DNA for 30 min with $10 \mu\text{g ml}^{-1}$ camptothecin (Fig. 1B) produces some conversion, an effect which can be heightened by increasing the concentration of camptothecin to $100 \mu\text{g ml}^{-1}$ (Figure 1C). As shown in Table 1, up to one-third of the component I molecules are converted to component II in these conditions. (Incubation without camptothecin causes no appreciable change in the distribution of components I and II.)

To characterise the mode of action of camptothecin further, viral DNA from treated and control cultures was centrifuged through alkaline sucrose gradients in conditions which allow the partial resolution of the separated circular (18S) and linear (16S) strands of component II (Fig. 2). Consistent

TABLE 1 Component I molecules converted to component II

Camptothecin treatment	% Component I	% Component II	% Component I converted to component II
None	67	33	—
$10 \mu\text{g ml}^{-1}$, 30 min	54	46	19
$100 \mu\text{g ml}^{-1}$, 30 min	44	56	34

Areas in Fig. 1 were determined under component I and component II peaks by the method of Girard, *et al.*¹³. Values are expressed as % of total component I plus component II.

with the results in Fig. 1, there is an increase in radioactivity in both the 18S and 16S peaks in the camptothecin-treated sample. (The amount of component I cannot be estimated as in these conditions some of the molecules pellet.) Quantita-

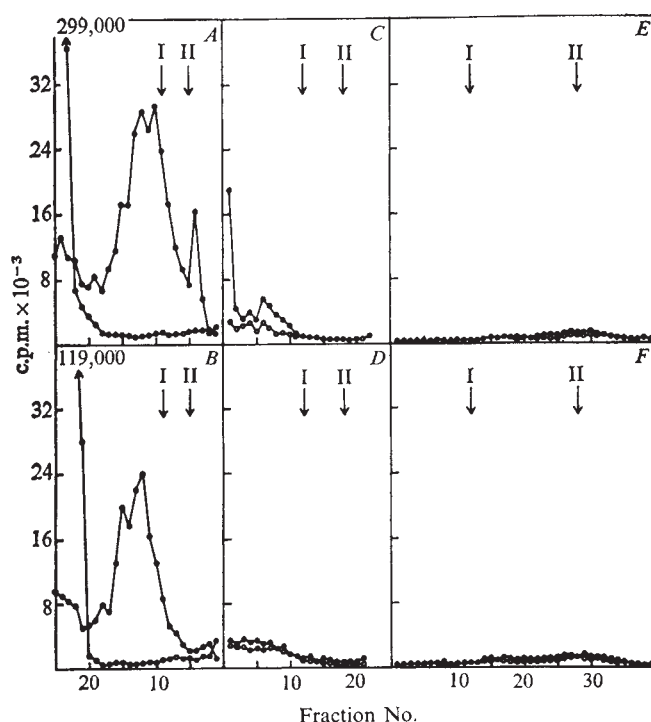


Fig. 3 Velocity sedimentation in alkaline gradients of cellular DNA from camptothecin-treated cells. Vero cells were labelled with $10 \mu\text{Ci ml}^{-1}$ ^3H -thymidine for the 12 h before infection. At 48 h post-infection, cultures were treated with $100 \mu\text{g ml}^{-1}$ camptothecin for 30 min, removed from the plates with trypsin-EDTA, and layered on alkaline sucrose gradients (A and B), as described by Abelson and Penman⁹. The 10 ml gradients containing 15–30% sucrose in 0.2 M NaOH, 1 M NaCl, 0.01 M EDTA, were prepared on top of a 0.5 ml cushion of 35% CsCl in the same buffer. Then 0.5 ml of lysing solution (1% Sarkosyl, 0.2 M NaOH, 5% sucrose) was layered on top of the gradient, followed by the suspension of whole cells. Parallel cultures were extracted by the method of Hirt¹². Aliquots (0.2 ml) of the Hirt supernatants were layered on the same type of gradients (C and D) or on alkaline CsCl (E and F). The alkaline sucrose gradients were centrifuged for 2 h at 40,000 r.p.m. in an SW 41 rotor at 24°C . Centrifugation of the alkaline CsCl was for 80 min at 44,000 r.p.m. in an SW 50.1 rotor at 15°C . The positions of the SV40 component I and II DNA added as external markers are indicated. A, C and E, DNA from infected cells; B, D and F, DNA from mock-infected cells. ●, camptothecin-treated cells; ○, untreated cells. Fractions were collected from the top using the Buchler Auto Densi-Flow (A and B), or from the bottom by puncturing the tube (C–F), then assayed on GF/B (A–D) or GF/A (E and F) filters (see legend to Fig. 1). In gradients displaying the centrifugation of aliquots of Hirt supernatants (C–F), the axes were corrected to represent the radioactivity present in the entire Hirt supernatant. Sedimentation is from right to left in all panels.

tion of the approximate areas under the 16S and 18S peaks demonstrates that they are in the same relative proportions in the camptothecin-treated sample as in the untreated control. This would not have resulted had the affected molecules received either a double-strand break or two single-strand breaks in opposite strands. In either case a preponderance of 16S linear molecules would have been observed.

Furthermore, there are no new lower molecular weight discrete species discernable due to drug treatment, as would be expected if the drug caused two or more breaks at specific sites on a single strand. It is possible, however, that the single-strand breaks caused by the drug are randomly located on the viral DNA. If this were the case, the result of multiple breaks would be relatively small increases in the ratio of 16S to 18S DNA, and in the heterodisperse, lower molecular weight background. We would not detect these changes in our experiments.

It might be argued that cellular DNA, which is broken or rendered alkali-labile by camptothecin treatment^{1,7-9}, would appear in the extracts, complicating our results. The patterns shown in Figs 1 and 2 give no indication that pulse-labelled cellular DNA, synthesised after infection, is extracted and sediments near viral DNA in alkaline conditions. We have also tested the effect of the drug on cellular DNA synthesised before the infection. The results presented in Fig. 3 show that pre-labelled cellular DNA is broken (or rendered alkali-labile) by the drug, and sediments in alkali only slightly faster than viral DNA (Fig. 3, A and B), but is removed or repaired during the extraction (Fig. 3, C-F).

We have shown that the principal change in SV40 DNA caused by camptothecin is the production of one single-strand break or alkali-labile region in the molecule. It is possible that additional breaks may occur at random locations in the molecule. These experiments with closed circular DNA constitute the first direct indication that the drug affects only one DNA strand, rather than inducing double-stranded alkali-labile regions.

It has still to be determined if the specificity of the camptothecin effect on SV40 DNA is due to the drug's preference for a particular sequence of nucleotides, similar to the mode of action of restriction endonucleases¹¹, or if the drug can cause a scission at any site on the molecule. In addition, it is still unclear whether the drug's effect on SV40 DNA is the induction of single-strand breaks or alkali-labile links, as has been suggested for cellular DNA by Abelson and Penman⁹. Further experiments are under way to determine the mechanism of action of camptothecin on SV40 DNA.

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Localisation of Monocyte Binding Site of Human Immunoglobulin G

THE binding of IgG to macrophages is thought to be important in both the afferent and efferent limbs of the immune response¹⁻³. Berken and Benacerraf⁴ studied the properties of guinea pig IgG2 antibodies cytophilic for macrophages and concluded that the binding site was in the Fc region. Similarly Lo Buglio *et al.*⁵ and Abramson *et al.*⁶ inhibited with isolated Fc fragment the binding of human monocytes to red cells coated with anti-D. Abramson *et al.*⁶ also studied the inhibitory activity of various fragments obtained by peptic digestion of IgG. The F(ab')₂ fragment was slightly inhibitory, unlike Fab fragment, and the authors suggested that the binding site was in the N-terminal portion of the Fc fragment (now known as the C_H2 region). Huber and Fudenberg⁷ reached similar conclusions following experiments in which F(ab')₂ fragments of anti-D antibodies were attached to red blood cells and produced rosettes with human monocytes, although the possibility of contaminating IgG could not be eliminated. In contrast, MacLennan *et al.*⁸ were unable to inhibit neutrophil phagocytosis of sensitised bacteria with immune complexes of antigen and the Facb fragment of rabbit IgG. Since the Facb fragment includes the Fab and C_H2 regions but lacks the C_H3 region this suggests that the latter may be the site of neutrophil binding. Furthermore, Yasmeen *et al.*⁹, working with a

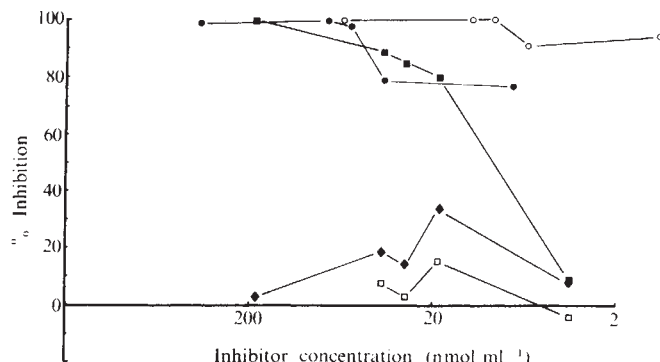


Fig. 1 Percentage inhibition of monocyte rosette formation obtained with different molar concentrations of IgG, various subfragments of IgG and human serum albumin. % Inhibition was obtained from the formula:

$$100 - \left[\frac{\frac{\text{Number of rosetting cells in test}}{\text{Number of monocytes in test}}}{\frac{\text{Number of rosetting cells in control}}{\text{Number of monocytes in control}}} \times 100 \right]$$

where the control consisted of cells and medium only. The curves represent values obtained in a single experiment. Similar values were obtained in a duplicate experiment. ○, Intact IgG; ●, pFc' fragment; ■, Fc fragment; ◆, Fab fragment; □, human serum albumin.

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heterologous system (human immunoglobulin G and guinea pig macrophages) have presented evidence that macrophage binding is a property of the C_H3 region of the molecule. In view of these conflicting reports we have investigated the possible role of the C_H3 region of human IgG in binding to homologous monocytes.

Human monocytes were prepared essentially as described by Hay *et al.*¹⁰ and resuspended to a final concentration of 4×10^5 cells ml⁻¹ in medium lacking foetal calf serum (FCS). The cell suspension ($0.5 \text{ ml} \equiv 2 \times 10^5$ monocytes) was distributed in equal quantities into chambers consisting of lucite rings affixed to 22 mm square glass coverslips. The chambers were placed in a humidified airtight box fitted with inlet and outlet tubes. The latter were used to introduce an 8% CO₂-air mixture into the box. After incubation for 1 h at 37° C each chamber was washed once with medium lacking FCS to remove cells not adhering to the walls of the chamber.

For the inhibition experiments Fc and Fab fragments of pooled human IgG (obtained from Kabi A. B., Stockholm) were prepared by papain digestion¹¹ and isolated by ion-exchange chromatography¹². The pFc' fragment of pooled IgG (i.e., the C_H3 region) was prepared by pepsin digestion and isolated by Sephadex G-150 gel filtration¹³. Similar series of different concentrations of each fragment were prepared (assuming E_{1,cm}^{1%} 280 nm of 14.6), and 0.5 ml of each preparation was added to a chamber. Every sample was duplicated and a control consisting of 0.5 ml of medium (without FCS) was also included. The chambers were flushed with 8% CO₂-air and incubated at 37° C for 1 h. The chambers were washed twice with medium and 0.5 ml of a 0.5% suspension of human red cells, previously coated with incomplete anti-D, was added to each. After flushing again with 8% CO₂-air the chambers were incubated at 37° C for 30 min and finally at 4° C for a further 30 min. Each chamber was then filled with medium, covered with a microscope slide and inverted to bring the coverslip uppermost. The degree of rosette formation was then assessed by phase contrast microscopy. Approximately 100 cells were counted in each chamber and monocytes binding two or more red cells were counted as rosettes.

Figure 1 shows the percentage inhibition of monocyte rosette formation obtained in a single experiment with different concentrations of pooled human IgG, Fc, Fab and pFc' subfragments of IgG and human serum albumin. All inhibitors were studied over the concentration range 0.02–1.0 mg ml⁻¹, but since the Fc and Fab fragments represent only one third, and the pFc' fragment one sixth, of the intact IgG molecule, the data in Fig. 1 are expressed on a molar basis. At concentrations between 20 and 200 nmol ml⁻¹ of each inhibitor the pFc' fragment, the Fc fragment and whole IgG showed levels of inhibition between 80% and 100% whereas HSA and Fab fragments showed values of less than 20%. Similar results were obtained over the same concentration range when the same protein preparations were tested in a second study. The percentage inhibition obtained with the different proteins and fragments (used at a concentration of 1 mg ml⁻¹, which discriminated readily between Fc and

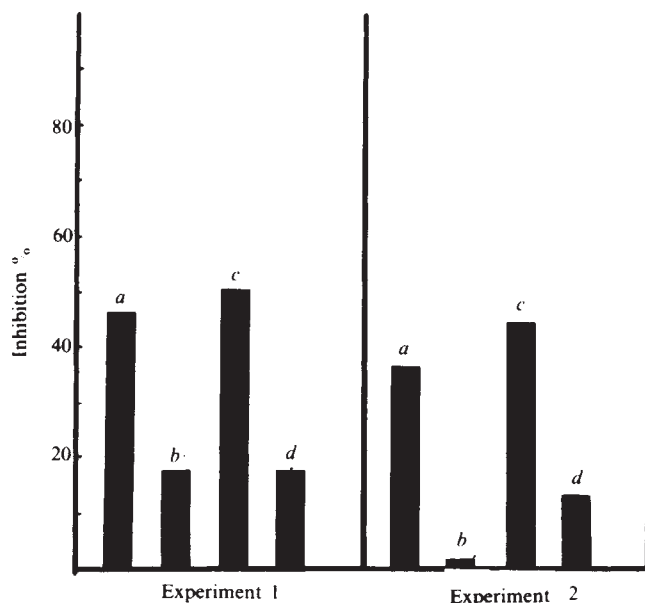


Fig. 2 Percentage inhibition of monocyte rosette formation obtained with preparations of pFc' fragment from, a, human IgG1; b, IgG2; c, IgG3; and, d, IgG4 myeloma proteins. Inhibitor proteins were prepared as previously described¹⁴ and detailed chemical characterisation is presented elsewhere¹⁵. The results of two separate experiments are plotted.

Fab fragments) in these and other replicate experiments are shown in Table 1. These results suggest that the binding site for homologous macrophage fixation is located in the pFc' region of the IgG molecule, as was also recently reported for heterologous interactions⁵.

In additional experiments inhibition of rosette formation was investigated with pFc' fragments isolated from all four subclasses of human IgG. Two IgG1 myeloma proteins and single samples of IgG2, IgG3 and IgG4 myeloma proteins were digested with pepsin to yield pFc' fragments and the fragments isolated by Sephadex G-150 gel filtration¹⁴. The results of two separate inhibition experiments are shown in Fig. 2. The pFc' fragments from IgG1 and IgG3 myeloma proteins inhibited rosette formation more strongly than did

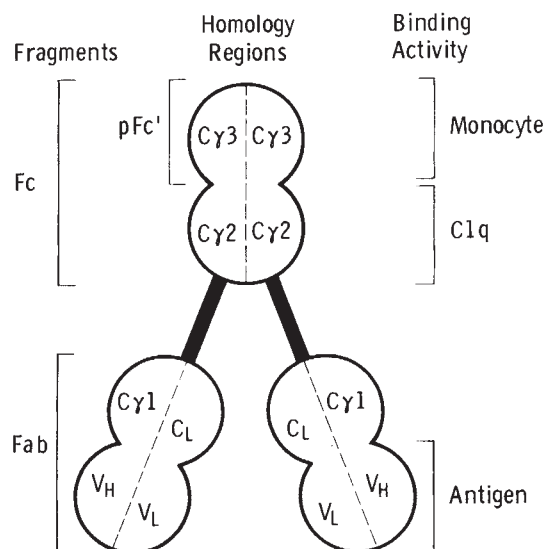


Fig. 3 Schematic model of human IgG molecule showing the binding activities associated with the different homology regions.

Table 1 Inhibition of Monocyte Rosette Formation by Pooled Human IgG and Various Subfragments

Inhibitor *	% Inhibition obtained in different experiments †		
IgG	100	100	100
Fc fragment	100	100	
Fab fragment	20	9	8
pFc' fragment	100	99	
Human serum albumin	30	2	

* All preparations used at 1 mg ml⁻¹ concentration.

† Calculated as in Fig. 1, and includes data from Fig. 1.

pFc' fragments from IgG2 and IgG4 myeloma proteins. The subclass distribution observed with pFc' fragments parallels exactly the results obtained using subclass proteins^{6,10,16}. None of the preparations, however, inhibited as well as pFc' fragment from pooled IgG. A possible explanation of this observation is that there are different binding sites for the different IgG subclasses, either on the same cell or on different cells. But such an explanation conflicts with the conclusions of Huber *et al.*¹⁶, who studied the monocyte binding activity of intact subclass proteins.

The possibility that the monocyte receptor interacts with aggregated rather than native pFc' fragment was investigated in the following manner. A concentrated preparation of pFc' fragment was chromatographed on Sephadex G-150 and a fraction of the eluted peak ($S_{20,w}=2.3$) was taken directly, without any concentrating procedure, for ultracentrifugal analysis in the MSE Centriscan at 60,000 r.p.m. Monocyte rosette inhibition was assayed simultaneously on the same sample. This material, which was shown to be fully inhibitory, contained no detectable aggregates (threshold of detectability 1.5% of total protein). Furthermore radio-labelled monomeric pFc' fragment was concentrated by three different procedures (ultrafiltration, lyophilisation and polyethylene glycol) and rechromatographed on Sephadex G-150. Aggregated material, if present, represented less than 0.25% of the total counts applied to the column. We therefore conclude that the monocyte receptor probably has specificity for native IgG rather than denatured or aggregated IgG.

Our study is further evidence for Edelman's 'domain concept'^{17,18} that the homology regions of the individual peptide chains of IgG interact to form six globular units each of which has evolved to perform a distinct function. Binding sites for antigen, for the C1q component of complement and for monocytes have now been localised to separate homology regions (see Fig. 3), and the importance of each of these activities might be expected to exert strong evolutionary pressure for the conservation of each binding site.

The ultracentrifugal analysis was simply performed by Drs D. R. Stanworth and P. Johns, University of Birmingham.

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Antibody response and virus survival in cats vaccinated against feline leukaemia

We have shown that leukaemia virus can be transmitted from cat to cat by contact or aerosol¹. There is also substantial epizootiological evidence that such transmission occurs in natural conditions^{2,3}. Horizontal transmission occurs because feline leukaemia virus (FeLV) replicates not only in cells of the haematopoietic system but is also found in large amounts in respiratory and alimentary mucous membranes and is excreted in the urine¹. It is therefore not surprising that the infection in cats is fairly common. In the City of Glasgow we found that about one third of the cats sampled had antibodies against FeLV (mean titre = 2; ref. 4). We recently examined 100 young cats from the surrounding countryside and found antibody in six (mean titre = 20). These facts indicate that vaccination would be valuable and we have evidence that it is feasible. Here we report on the use of FeLV-infected cells to produce an immune response of a high level in cats.

The antibodies being measured are directed against the virus subgroup specific glycoprotein antigens which are located on the membrane of the infected cell and on the envelope of the virus; they have the same specificity as those responsible for virus neutralisation and cell cytotoxicity (W. J., L. M., O. J., H. L., and C. H., unpublished). An indirect immunofluorescence test is used in which the target cells are living, virus-infected, feline lymphoblasts which are cultured in suspension^{4,5}.

We inoculated cats with infected cells for two main reasons. First, in the feline sarcoma virus (FeSV) system, high antibody titres have been associated with either failure of tumour induction or regression of established tumours⁶. Similar results have not been reported in feline leukaemia. We therefore tried to mimic the sarcoma situation by injecting cats with packs of fibroblasts which were actively replicating FeLV from their cell membranes. This was on the assumption that to obtain high antibody levels it might be necessary to present cell walls bearing antigen arrays to

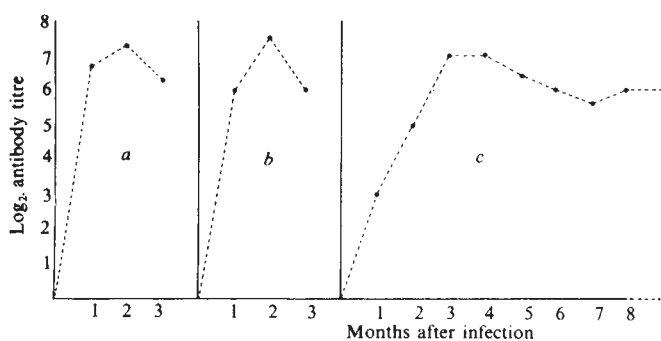


Fig. 1 Group mean antibody responses in three groups of cats inoculated with FeLV-infected cells. Group a received 4×10^7 feline embryo (FEA) monolayer cells; group b were given 4×10^7 feline lymphoblastoid cells grown in suspension, and group c received 2×10^9 FEA cells.

TABLE 1 Type and number of cells infected with various subgroups of FeLV used to immunise cats, the peak antibody titres induced the relationship of the immune response to the establishment or persistence of virus infection

Cat	Type	Cells No.	Peak anti-body titre	Duration of experiment (months)	Virus isolation after necropsy	Virus subgroups
1	FEA*	2×10^9	256	13	+	A + B
2	FEA	2×10^9	128	13	+	A + B
3	FEA†	2×10^9	32	6	+	A + B
4	FEA‡	2×10^9	64	6	+	A + B
5	FEA§	2×10^9	256	6	+	A
6	FEA§	7×10^8	128	6	+	A
7	FEA§	7×10^8	64	6	+	A
8	FEA	4×10^7	128	1	0	A + B + C
9	FEA	4×10^7	32	1	0	A + B + C
10	FEA	4×10^7	16	1	0	A + B + C
11	FEA	4×10^7	128	1	0	A + B + C
12	FEA	4×10^7	256	3	0	A + B + C
13	FEA	4×10^7	32	3	0	A + B + C
14	FEA	4×10^7	512	3	0	A + B + C
15	FEA	4×10^7	512	3	0	A + B + C
16	FL†	4×10^7	16	1	0	A + B + C
17	FL	4×10^7	128	1	0	A + B + C
18	FL	4×10^7	512	1	0	A + B + C
19	FL	4×10^7	64	3	0	A + B + C
20	FL	4×10^7	256	3	0	A + B + C
21	FL	4×10^7	512	3	0	A + B + C

Cats were approximately 6 months old at the start of the experiment and had no demonstrable specific antibody at that time.

* FEA, feline embryo fibroblasts grown as monolayers.

† FL, feline lymphoblasts grown in suspension.

‡ Simultaneous administration of 4×10^{10} *B. pertussis*.

§ Simultaneous administration of 4×10^{10} *B. pertussis* and 1 ml Freund's complete adjuvant.

the immune system. Second, we considered it likely that the integrity of the glycoprotein complexes which are the effective immunogens might be well preserved by this presentation.

Feline embryonic fibroblast cells of the FEA strain were grown as monolayers (FE) and were infected with FeLV as previously described⁷; isolates containing various FeLV subgroups (A; A + B; and A + B + C) were used⁸. Cells were collected, washed three times in Alsever's solution and pelleted by low speed centrifugation. Minimal buffer was added to enable the cell pack to pass through an 18 gauge needle for subcutaneous injection.

Serum samples were taken before inoculation and afterwards at monthly intervals. Detailed haematological examinations were made to check for the possible development of anaemia, leukaemia or the thymic deficiency and immunosuppression syndrome, all of which are caused by FeLV infection. When the animals were killed they were examined for the presence of FeLV by two methods. Preparations of bone marrow and tracheal epithelium, the two most sensitive monitoring sites¹, were examined electron microscopically (EM). Bone marrow cells were also co-cultivated with FE cells growing as monolayers. The latter were then subcultured every 3 to 4 d for 21 d. The cells were divided; one aliquot was used to prepare sections for EM examination and the other was examined for the presence of FeLV group specific antigen by an indirect immunofluorescence test using goat anti-FeLV antiserum¹.

The results are shown in Table 1. When 2×10^9 cells were given, high antibody titres resulted and these persisted for at least 13 months. It will be seen from Fig. 1c that these cats did not reach their maximum antibody titre for 3 months; after they were killed, virus was found in the bone marrow. In contrast, cats which were inoculated with 4×10^7 cells (cats 8 to 15), produced good antibody response by 1 month after inoculation (Fig. 1a) and no virus was found in tissues taken at necropsy. At no time did any of these animals show a clinical or haematological abnormality

and no lesions were seen at necropsy or found on histological examination.

This experiment was repeated (Table 1, cats 16 to 21) using infected feline lymphoblasts grown in suspension culture; again a mean antibody titre of 128 was obtained. Virus was not detected at autopsy 1 or 3 month after inoculation and the cats remained healthy throughout. It is obvious from Fig. 1a and b that a rapid antibody response occurred in the first month to near peak titre. It is not known if these cats became infected during the first month or if the infection was suppressed by the rapidly rising antibody level. We are currently carrying out a larger experiment to allow weekly monitoring for infection during the first month. It would appear, however, that it is possible to obtain high titres using infected cells. In the small number of cases in which adjuvants were incorporated (Table 1) there was no apparent benefit and the peak antibody titre was not reached until the 4th month.

We have found that if cats which have naturally acquired specific antibody are inoculated with infected cells or purified virus, a secondary response results. The animal in Fig. 2a, which was typical of several, had a naturally occurring titre of 16. It was inoculated with 4×10^7 FeLV-infected FE cells and showed a rapid and high response. Virus could not be detected after autopsy. Another case from a different experiment is shown in Fig. 2b. This cat had a preinoculation titre of 8. It was given intravenously a purified FeLV preparation containing approximately 10^{10} virus particles when it was 4 months of age. There was viraemia between months 3 and 7, but during this period there was an antibody rise. The animal remained healthy until killed; there was no demonstrable virus in samples taken at autopsy. At the start of this experiment, none of the other nine animals involved had antibody. All became infected and three developed leukaemia during the limited time span of the experiment. The low antibody response of this group is shown in Fig. 2b.

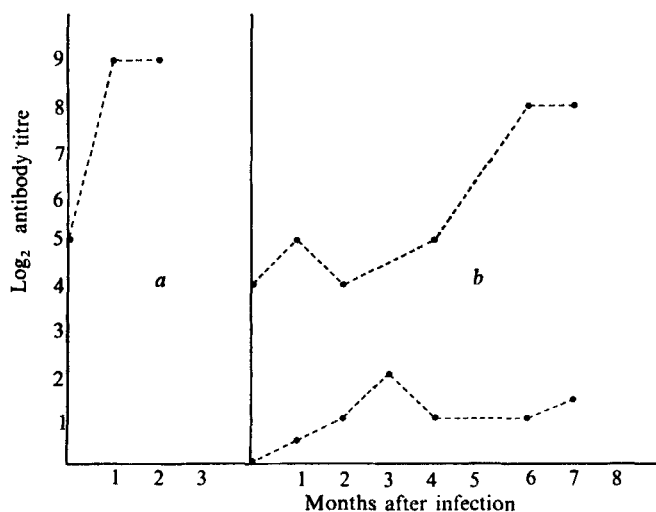


FIG. 2 a, This young adult cat had 5 antibody units acquired by natural exposure to infection. It was inoculated with 4×10^7 FeLV-infected FEA cells subcutaneously. A high antibody titre was found at 1 month. No virus could be detected when the animal was killed after 2 months. b, A young adult cat had 4 naturally acquired antibody units. It was infected with a high dose of gradient purified virus which is normally 100% pathogenic. Viraemia was present after 3 months. Virus then disappeared and none was found at autopsy. At this time a high antibody level was present. c, Gives the mean antibody response of the other nine cats in this experiment. None had antibody titres before inoculation with virus and all became infected; three developed leukaemia before the experiment was terminated.

The following conclusions may be drawn from these results. If the cat had previous experience of FeLV and had responded immunologically even to a mild degree, a secondary response occurred and no persistent infection resulted. If the virus input dose was high, the animal became infected but subsequently suppressed the infection. Cats with no previous experience of infection seemed to have a cell dose-related response. The higher doses induced a good antibody level but this did not terminate the infection, even when observed over a long period. The persistence of infection may have been associated with the slower rate of antibody rise and its inability to prevent the establishment of infection of epithelial cells of many tissues¹. These cells may not be susceptible to the action of cytotoxic antibody. In the groups given lower doses the antibody rise took place in the first month and virus was not found in the animals at that time or later.

The mean antibody levels achieved in these experiments are considerably higher than those seen in cats with naturally acquired infections. The highest titre we have seen in a spontaneous feline leukaemia case was 32, and this was exceptional. Seventy-five per cent of cases had no demonstrable antibodies and the mean level of those which had was 8. In our surveys of normal cats, the highest titre seen in those which had antibodies was 64 and the mean was 4.

These results indicate the feasibility of vaccination against leukaemia by inducing either large primary responses or small primary and large secondary responses. As leukaemia and its associated diseases are not usually found in immature cats and as most animals seem to become infected horizontally by contact later in life^{1,2}, the time scale of the natural history of the disease is suitable for the application of immunisation. We have already obtained significant antibody levels with inactivated vaccines; these experiments will be reported later.

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(con A) on mouse lymphoid cells is also accompanied by considerable endocytosis of label^{3,4}. On the other hand, little or no internalisation of label occurs after capping of H-2^{1,3} and Thy-1 (0)¹ alloantigens on mouse lymphocytes and thymocytes, and anti-lymphocyte globulin receptors on rat spleen cells³.

To better define the relationship between cap formation and endocytosis, we examined capping of surface Ig, con A receptors, and H-2, Thy-1 and TL alloantigens on mouse thymus and spleen cells by immuno-electron microscopy and immunofluorescence. We report that caps form primarily in two distinct locations on the cell surface, either over or directly opposite the Golgi region. Location of capping seems to be determined more by cell type than by surface receptor, with caps generally forming over the Golgi region of spleen cells, and opposite the Golgi region of thymus cells. Caps over the Golgi region are extensively internalised by endocytosis, but there is little or no internalisation of caps forming opposite the Golgi region. Furthermore, cap formation over the Golgi region was partially inhibited by cytochalasin B, while capping opposite the Golgi was unaffected.

For these studies, mice of the C57Bl/6/TL+ congenic stock were used. Suspensions of thymus and spleen cells with greater than 90% viability (determined by trypan blue exclusion) were prepared in medium 199 supplemented with 5% (v/v) gamma globulin-free foetal bovine serum, and cells were incubated and washed in this medium. All incubations were for 30 min at 37° C, with cells maintained in suspension by periodic agitation. Cells were washed twice by centrifugation at 4° C after each incubation.

The following mouse alloantisera were used to label H-2, Thy-1 and TL alloantigens: (C57Bl/6/H-2^k × A)_{F1} anti-C57Bl ascites leukaemia EL4 (anti-H-2^b), (A/Thy-1.1 × AKR/H-2^b)_{F1} anti-A strain spontaneous leukaemia ASL1 (anti-Thy-1.2), and (A/TL- × C57Bl/6)_{F1} anti-ASL1 (anti-TL.1,2,3).

Alloantigens on thymus cells were labelled for immuno-electron microscopy by incubating cells sequentially with the

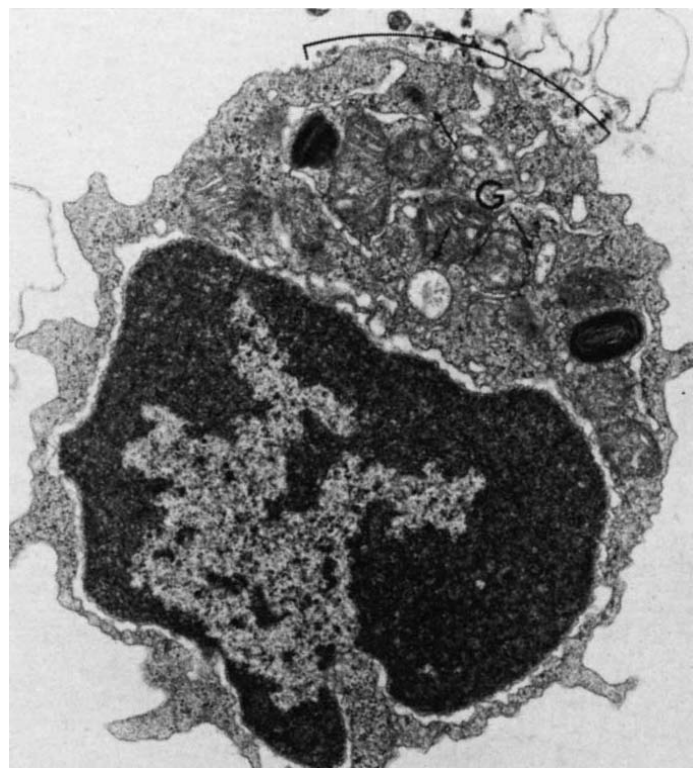


Fig. 1 Section through the approximate centre of a spleen cell, with a cap of con A receptors, labelled with con A, hybrid anti-con A/anti-ferritin antibody, and ferritin, directly over the Golgi region (G). The cap of ferritin is marked by a bracket, and label internalised in vesicles is indicated by arrows. (×17,100.)

Two Distinct Types of Capping of Surface Receptors on Mouse Lymphoid Cells

IMMUNOGLOBULIN (Ig) molecules on the surfaces of metabolically active mouse lymphocytes are redistributed into "caps" when crosslinked with fluorescent, ferritin-conjugated or ¹²⁵I-labelled anti-Ig antibody at room temperature or 37° C¹⁻³. Caps of labelled Ig-anti-Ig complexes form over the Golgi region of the cell, and are rapidly ingested by endocytosis^{2,3}. Capping of receptors for the plant lectin concanavalin A

Table 1 Site of Cap Formation and Amount of Endocytosis of Cell Surface Receptors

Receptor	Cell	Over Golgi	No. of caps		Endocytosis of label
			Opposite Golgi	Intermediate	
Surface immunoglobulin	Spleen	28	0	2	++++
Con A receptors	Spleen	10	2	0	++++
H-2 alloantigen	Thymus	3	12	5	±
Thy-1 alloantigen	Spleen	10	11	8	++
	Thymus	1	9	2	±
	Spleen	12	2	0	+++
TL alloantigen	Thymus	2	18	5	-

appropriate alloantiserum (anti-H-2 and anti-Thy-1 were diluted 1 : 10, and anti-TL 1 : 100), then with the hybrid rabbit F(ab')₂ antibody, anti-mouse IgG/anti-ferritin⁴, and finally with ferritin. Hybrid antibody induces cap formation in the same manner, and nearly as effectively, as bivalent antibody⁵. Hybrid antibody directed against the Fc fragment of mouse IgG (anti-mouse Fc/anti-ferritin) was used on spleen cells, to discern applied alloantibody from naturally-occurring surface Ig⁶. Surface Ig was labelled with hybrid anti-mouse IgG/anti-ferritin and ferritin, and con A receptors with purified con A⁷ (100 µg ml⁻¹), hybrid anti-con A/anti-ferritin antibody, and ferritin. Hybrid antibodies and ferritin were used at a concentration of 100 µg ml⁻¹.

For immunofluorescence, alloantigens were labelled with alloantiserum and either 300 µg ml⁻¹ of fluorescent rabbit anti-mouse IgG (on thymus cells) or 250 µg ml⁻¹ of fluorescent rabbit anti-Fc fragment of mouse IgG (on spleen cells). Surface Ig was labelled with fluorescent rabbit anti-mouse IgG (130 µg ml⁻¹), and con A receptors with con A and fluorescent rabbit anti-con A (200 µg ml⁻¹). The purified IgG fraction of rabbit antiserum was coupled to fluorescein isothiocyanate by the method of Cebra and Goldstein⁸.

To determine the site of cap formation relative to the Golgi region of the cell, and to estimate the amount of internalisation of label, cell suspensions were first labelled for immuno-electron microscopy and then allowed to settle onto a carbon-coated glass surface before preparation for electron microscopy. We found previously⁹ that lymphoid cells usually settle onto a surface with the Golgi region oriented to one side. Serial sections were cut parallel to the plane of the monolayer, from which sections through the approximate centre of the cell, including the Golgi region, were selected for analysis. The approximate amount of endocytosis of label was estimated from the number of cytoplasmic vesicles containing ferritin. Results are presented in Table 1.

Surface Ig and con A receptors capped over the Golgi region on 93% and 83%, respectively, of spleen cells analysed, and the caps were extensively internalised (Fig. 1). In contrast, H-2, Thy-1 and TL alloantigens capped over the Golgi region on only 15%, 9% and 8% of thymus cells, respectively. Caps on these cells formed predominantly directly opposite the Golgi region, and very little endocytosis of label was observed (Fig. 2). Although Thy-1 alloantigen capped primarily opposite the Golgi region on thymus cells, 86% of caps of the same alloantigen on spleen cells formed over the Golgi region, and there was considerable endocytosis of label. These results suggest that the cell type, rather than the receptor, determines the cellular site of cap formation, and that the amount of endocytosis depends on the site of cap formation. Capping of H-2 alloantigen on spleen cells was not polarised, and endocytosis occurred to a moderate extent, primarily in cells with caps in the vicinity of the Golgi region. Capping of con A receptors on thymus cells occurs too infrequently for accurate determination of the predominant site of cap formation by immuno-electron microscopy.

We attempted to differentiate further these two types of cap by testing the effects of inhibitors of active cell movements on

cap formation. These results, obtained by immunofluorescence, are summarised in Table 2. Sodium azide, which may suppress cell movements by blocking intracellular ATP formation¹⁰, inhibited capping of all surface receptors to a similar extent. However, cytochalasin B, which prevents cell locomotion by disrupting cytoplasmic contractile microfilaments¹¹, strongly inhibited capping of surface Ig, con A receptors and Thy-1 alloantigen on spleen cells, but had only a slight inhibitory effect on capping of H-2 alloantigen on spleen cells, and virtually no effect on capping of the other surface receptors. Inhibition of cap formation was readily reversed by incubating treated cells in medium lacking cytochalasin B.

Since cytochalasin B was dissolved in dimethyl sulphoxide (DMSO) before use (100 µg ml⁻¹ in medium 199 containing 10% DMSO), DMSO at the same concentration used in conjunction with cytochalasin B was tested alone for its effects on capping. Results indicate that the differential effect of cytochalasin B on cap formation is not due to the DMSO vehicle. Furthermore, although cytochalasin B has been shown to suppress glucose uptake by cells¹², the observed inhibition

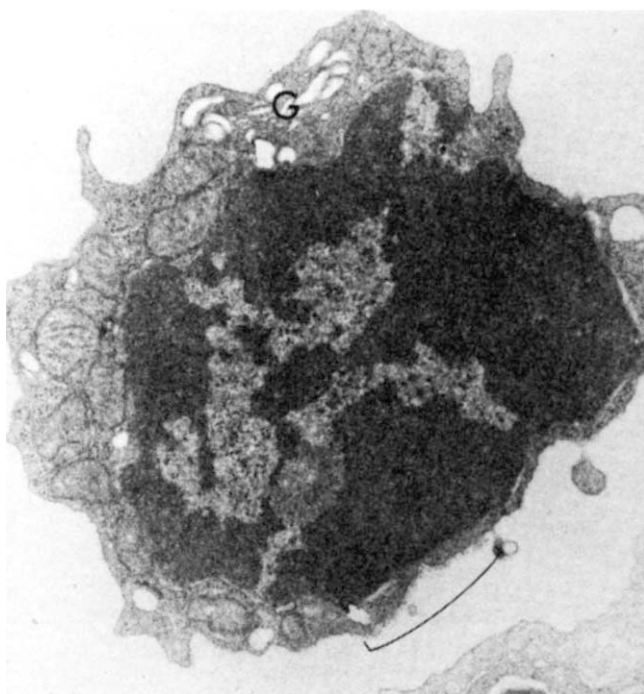


Fig. 2 Section through the approximate centre of a thymus cell, with a cap of TL alloantigens, labelled with anti-TL alloantiserum, hybrid anti-mouse IgG/anti-ferritin antibody, and ferritin, directly opposite the Golgi region (G). The cap of ferritin is marked by a bracket. No internalisation of label is evident. ($\times 17,100$)

Table 2 Effect of Inhibitors of Active Cell Movements on Cap Formation

Receptor	Cell	Sodium azide (10^{-1} M)	% Inhibition of capping*			
			Cytochalasin B (5×10^{-4} M)	DMSO (10^{-1} M)	Vincristine (5×10^{-4} M)	Vinblastine (5×10^{-5} M)
Surface immunoglobulin	Spleen	75	57	7	3	4
Con A receptors	Spleen	86	81	3	0	2
H-2 alloantigen	Thymus	80	7	6	2	0
	Spleen	71	15	8	6	5
Thy-1 alloantigen	Thymus	87	0	2	0	4
	Spleen	86	54	4	5	4
TL alloantigen	Thymus	86	6	6	1	0

* Cells were incubated with the appropriate inhibitor for 15 min at 37° C before immunofluorescence labelling in the continuous presence of the inhibitor. Inhibitors were used at the maximum concentrations at which 80% or more of the treated cells remained viable when labelling was completed. Each figure represents an average of three or more determinations.

of capping is apparently not due to impaired glucose uptake, since labelling of cells in medium lacking glucose had no effect on capping of surface receptors on either thymus or spleen cells.

Vincristine and vinblastine, two anti-mitotic alkaloids which precipitate microtubule proteins¹³, have no significant inhibitory effect on either type of cap formation (Table 2). Colchicine, colcemid and strychnine, drugs which have related effects on cellular microtubules¹⁴, also had no effect on either type of capping when used at a concentration (5×10^{-4} M) at which 80% or more of the treated cells remain viable.

The sensitivity of capping over the Golgi region of spleen cells to cytochalasin B indicates that this type of cap may form as a result of active cell movements mediated by a system of cytoplasmic contractile microfilaments. Capping opposite the Golgi region of thymus cells apparently does not result from the activity of contractile microfilaments or microtubules. Nevertheless, inhibition of this type of capping by sodium azide suggests that some sort of active cell movements is involved. Capping over the Golgi region and extensive endocytosis of capped label may require cell movements controlled by a system of contractile microfilaments, with capping opposite the Golgi region occurring in the absence, or during inactivity, of such a system. The lack of polarity, and relative insensitivity to cytochalasin B, of H-2 alloantigen capping on spleen cells may, however, indicate that there are additional factors involved in determining the cellular site of cap formation.

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T cell factor which can replace T cells in vivo

THE mechanism whereby thymus-derived (T) and bone marrow or bursa-derived (B) lymphocytes cooperate in the induction of an antibody response is of great importance in immunology. It has been suggested that T cells concentrate antigen by their surface receptors to present a multideterminant antigen array directly to B cells^{1,2}; or that T cells secrete a special class of cooperating antibody (IgX) for this purpose, which could specifically present antigen at the surface of a third cell, the macrophage^{3,4}; or that the product of recognition of antigen by T cells has some non-specific mitogenic or stimulatory effect on B cells^{5,6}. Recent work with *in vitro* systems has indicated that various factors produced by T cells, either after contact with a specific antigen or following stimulation by allogeneic cells, can replace the requirement for T cells in antibody formation⁷⁻¹⁴.

Here, I describe the preparation of a T cell factor capable of totally replacing T cells *in vivo* in the induction of an antibody response to a highly thymus dependent synthetic polypeptide antigen, (T,G)-A--L. The factor is prepared *in vitro* by incubation of primed or 'educated' mouse T cells with antigen for 6-8 h; cells are then removed by centrifugation and the supernatant containing the factor is mixed with

TABLE 1 Activity of a T cell factor *in vivo*

Matter transferred into irradiated recipients	Antigen	PFC ($\pm$ s.e.)
B cells*	(T, G)-A--L	124 ($\pm$ 28)
B cells + normal T cells†	(T, G)-A--L	4,360 ($\pm$ 320)
B cells + T cells educated to (T, G)-A--L‡	(T, G)-A--L	5,100 ($\pm$ 364)
B cells + supernatant produced by T cells educated to (T, G)-A--L§	(T, G)-A--L	4,850 ($\pm$ 260)

* 10^7 bone marrow cells.

† 10^8 thymocytes.

‡ 1 spleen equivalent (about 5×10^6 cells).

§ Produced by 1 spleen equivalent educated T cells.

The direct anti-(T, G)-A--L plaque forming cell response in irradiated (850 r.) Balb/c mice 12 days after receiving transfer of B cells, or B cells mixed with normal or educated T cells or a T cell-produced supernatant factor, and (T, G)-A--L. Results as geometric means of 15 mice $\pm$ s.e.

TABLE 2 Conditions of *in vitro* culture for the preparation of a T cell factor

Cells present in culture <i>in vitro</i>	Antigen (<i>in vitro</i>)	PFC(±s.e.)*
Normal T cells†	(T, G)-A--L	116(±38)
T cells educated to sheep red blood cells‡	(T, G)-A--L	84(±16)
T cells educated to (T, G)-A--L‡	—	204(±42)
T cells educated to (T, G)-A--L	Sheep RBC	213(±26)
T cells educated to (T, G)-A--L	(T, G)-A--L	4,200(±480)

† 1 thymus equivalent per ml medium.

‡ 2 spleen equivalents per ml medium.

* Direct anti-(T, G)-A--L PFC measured 12 d after transfer of supernatant with 10⁷ bone marrow cells and 10 µg (T, G)-A--L into irradiated recipients.The direct anti-(T, G)-A--L plaque forming cell response in irradiated (850 r.) Balb/c mice 12 days after receiving transfer of syngeneic B cells mixed with (T, G)-A--L and supernatants produced *in vitro* by different combinations of normal or educated T cells and antigen. Results as geometric means of 15 mice ± s.e.

normal bone marrow cells and antigen and transferred into lethally irradiated recipients. Under these conditions antibody-forming cells are produced in the spleens of the recipients in numbers identical with those produced by mixtures of T cells, B cells and antigen. Moreover, the T cell factor has been shown to be completely specific for the inducing antigen.

Experiments used Balb/c mice, bred at the Department of Pathology, Cambridge. The antigen was the multichain synthetic polypeptide poly-(Tyr, Glu)-poly-DL Ala--poly-Lys, abbreviated as (T,G)-A--L, and was the gift of Dr Edna Mozes.

T cells were 'educated' or primed to (T,G)-A--L by transfer of 10⁸ Balb/c thymocytes into lethally irradiated (850 rad) syngeneic recipients, followed 1 d later by injection of 10 µg (T,G)-A--L in complete Freund's adjuvant. The spleens of these recipients, containing the educated T cells, were removed 7 d later, and contained approximately 5 × 10⁶ viable lymphocytes per spleen by the criterion of trypan blue exclusion. Cell suspensions from the spleens were prepared in minimal Eagle's medium (Flow) with no further additions of protein or nutriment, with 10 spleen equivalents of cells per 5 ml of medium. (T,G)-A--L to a final concentration of 1 µg per ml was added and the cells incubated in small Petri dishes (Sterilin P 122, 50 mm × 13 mm) at 37° C, under an atmosphere of 10% CO₂. The type of Petri dish used is important, since dishes of different grade and manufacture (Falcon Tissue Culture Dishes) gave very unsatisfactory results.

Incubation was continued for 6–8 h, after which the cell suspensions were transferred to a centrifuge tube and the cells spun down. The cell-free supernatant was tested for its ability to cooperate with B cells by mixing with bone marrow

cells and antigen and transferring into lethally irradiated syngeneic recipients. Each recipient received supernatant equivalent to 1 spleen of educated T cells, 10⁷ bone marrow cells, and 10 µg of (T,G)-A--L dissolved in saline, inoculated into a tail vein. Suitable controls were included as described below.

Twelve days later the spleens of these animals were removed and direct plaque-forming cells (PFC) to (T,G)-A--L determined. For the plaque assay, sheep red blood cells were coated with (T,G)-A--L by mixing equal volumes of packed sheep erythrocytes, chromic chloride (10 mg ml⁻¹), and (T,G)-A--L (2 mg ml⁻¹). After 5 min at room temperature the coated cells were spun down and washed three times. The results shown in the tables are geometric means ± s.e. with 15 animals per group.

Table 1 shows that the supernatant produced by T cells educated to (T,G)-A--L was able to cooperate with B cells when transferred together into irradiated recipients. The level of the plaque forming response was very much higher than that given by B cells alone, and not significantly different from the response of B cells and 10⁸ normal or 5 × 10⁶ educated T cells. Thus, after a relatively short period of incubation with antigen *in vitro*, educated T cells released a factor or factors capable of replacing T cells in a cooperation-dependent antibody response *in vivo*. The conditions required for production of the factor *in vitro* are shown in Table 2. There was an absolute requirement both for educated T cells and the inducing antigen. Neither normal thymocytes, nor T cells primed against a non-cross-reacting antigen (sheep red blood cells) released any significant cooperating activity when incubated *in vitro* with (T,G)-A--L; and T cells educated specifically to (T,G)-A--L only released factor in the presence of (T,G)-A--L.

The specificity of action of the supernatant factor produced to (T,G)-A--L was investigated by transfer together with B cells and a non-cross-reacting thymus-dependent antigen, sheep red blood cells. Table 3 shows that the enhancing activity of the supernatant was specific for (T,G)-A--L and was completely without effect on the response to sheep red blood cells.

The possibility of contamination of educated T cells by B cells must be considered. No anti-(T,G)-A--L PFC were present in the educated T cell preparations, and no amount of conventional anti-(T,G)-A--L antibody tested could replace the requirement for T cells or T-cell factor *in vivo* (personal observation). To demonstrate conclusively that T cells were responsible for the production of the factor, educated T cells were treated with mouse anti-θ serum and complement before culture with antigen *in vitro*. (Anti-θ serum, raised in AKR mice to CBA thymocytes, was the gift of Dr A. Munro.) After treatment with anti-θ serum (or normal mouse serum as control) at a dilution of 1:2 for 30 min, the educated T cell preparation was washed and treated in the cold with guinea pig complement, previously

TABLE 3 The specificity *in vivo* of a T cell factor produced *in vitro* to (T, G)-A--L

Matter transferred into irradiated recipients	Antigen	PFC (± s.e.)	
		Anti-SRBC	Anti-(T, G)-A--L
B cells*	SRBC	130 (±33)	
B cells + normal T cells†	SRBC	2,561 (±210)	
B cells + T cells educated to (T, G)-A--L‡	SRBC	116 (±48)	
B cells + supernatant produced by T cells educated to (T, G)-A--L§	SRBC	84 (±26)	
B cells	(T, G)-A--L		250 (±46)
B cells + supernatant produced by T cells educated to (T, G)-A--L	(T, G)-A--L		3,970 (±384)

* 10⁷ bone marrow cells.† 10⁸ thymocytes.‡ 1 spleen equivalent (about 5 × 10⁶ cells).

§ Produced by 1 spleen equivalent educated T cells.

The direct anti-sheep red blood cell (SRBC) and anti-(T, G)-A--L plaque forming cell responses in irradiated (850 rad) Balb/c mice 12 d after receiving transfer of different combinations of syngeneic B cells, antigen, and either T cells or a supernatant factor produced *in vitro* by T cells educated to (T, G)-A--L. Results as geometric means of 15 mice ± s.e.

TABLE 4 Effect of anti- θ serum on the ability of educated T cells to produce cooperating factor

Treatment of educated T cells before culture <i>in vitro</i>	Antigen	PFC($\pm$ s.e.)
None	(T, G)-A--L	3,812($\pm$ 486)
Normal mouse serum and complement	(T, G)-A--L	2,940($\pm$ 260)
Anti- θ serum and complement	(T, G)-A--L	282($\pm$ 106)

T cells educated to (T, G)-A--L were treated with anti- θ serum and complement, or normal mouse serum and complement, before culture with (T, G)-A--L *in vitro*. The factors produced by the surviving cells cultured with (T, G)-A--L *in vitro* were transferred together with B cells and (T, G)-A--L into irradiated Balb/c recipients. The results show the direct anti-(T, G)-A--L plaque forming cells 12 d after transfer. Results as geometric means of $15 \pm$ s.e.

absorbed with agarose and at a dilution of 1:10. Dead cells were subsequently removed by passage through a cotton wool column in low ionic strength buffer after the method of Von Boehmer and Shortman¹⁵. The effluent cells were restored to normal buffer conditions and cultured *in vitro* with antigen for 8 h. Table 4 shows that cells treated with anti- θ serum and complement were unable to produce factor to (T, G)-A--L *in vitro*, whereas controls treated with normal mouse serum and complement showed undiminished ability to produce the active factor.

The cooperating factor described can be compared with that described by Feldmann and coworkers^{4,7}, which is produced by activated T cells *in vitro*, is active in collaborating with B cells *in vitro*, and is also antigen-specific in its induction and action. Its *in vivo* properties have not been reported. On the other hand, there is a clear contrast with various non-specific enhancing factors produced by T cells in response to to allogeic stimuli^{8-10,12,13} or other strong antigenic stimuli¹⁴.

It is possible that under physiological conditions *in vivo*, the joint action of two factors is required to initiate an antibody response in B cells. The first would be a T-cell product endowed with specificity—presumably through an antibody-like combining site—which could present the antigen in a multivalent fashion to B cells, for example by effectively polymerising antigen at the macrophage surface^{3,4}. The high strength of binding to B cells achieved thereby would be one prerequisite for effective antigen recognition by B cells and for B cell triggering. The other requirement would be a non-specific mitogenic stimulus provided by a second T-cell product, needed to send the B cell into proliferation. An excess of the latter product—as produced by allogeic stimuli (the 'allogeic effect')^{5,6,13}—could override the requirement for the former and for multivalent binding of antigen to B cells, by providing unusually high mitogenic stimulation. Thymus-independent antigens would be expected to possess multiple antigenic determinants of the same specificity, and in addition to have an independent mitogenic effect on B cells^{16,17}, thus circumventing T cells altogether.

Studies are in progress to identify the factor described here and in particular to determine whether it comprises more than one active component.

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Demonstration of suppressor T cells in a population of 'educated' T cells

THE regulation of the immune response, in the sense of the limitation of its extent, can probably be achieved in two main ways. The first, and more extensively studied, is the feedback effect of the antibody end-product, brought about either by the removal of antigen, or by the blockage of antigen recognition by antigen-sensitive cells¹. A second possibility suggested more recently is that certain regulatory cells exist which can exert an actively suppressive influence on immune responses². Since they are believed to be among the thymus-derived or T cell population, they may be termed suppressor T cells. Evidence for their existence includes the enhancement of certain responses by anti-lymphocyte serum³⁻⁵, the suppressive influence of graft-versus-host reactions^{6,7}, and recent observations on antigenic competition^{8,9}, infectious tolerance¹⁰, and allotype suppression¹¹.

TABLE 1 Cooperative activity *in vivo* of (T, G)-A--L-educated T cells, before and after culture *in vitro* with (T, G)-A--L

Cells transferred into irradiated recipients	Antigen (<i>in vivo</i>)	PFC $\pm$ s.e.
B cells*	(T, G)-A--L	186 $\pm$ 28
B cells + normal T cells†	(T, G)-A--L	3972 $\pm$ 416
B cells + (T, G)-A--L-educated T cells, before culture <i>in vitro</i> ‡	(T, G)-A--L	5621 $\pm$ 403
B cells + (T, G)-A--L-educated T cells, after culture <i>in vitro</i> without antigen.	(T, G)-A--L	4214 $\pm$ 318
B cells + (T, G)-A--L-educated T cells, after culture <i>in vitro</i> with (T, G)-A--L	(T, G)-A--L	0

* 10^7 bone marrow cells.

† 10^8 thymocytes.

‡ 5×10^6 viable cells.

The direct anti-(T, G)-A--L plaque forming cell (PFC) responses in irradiated Balb/c mice 12 d after receiving transfer of B cells, or B cells mixed with normal or (T, G)-A--L-educated T cells, and (T, G)-A--L. The treatment of the educated T cells before transfer is indicated. Results as geometric means of 15 mice $\pm$ s.e.

TABLE 2 Suppressive activity of (T, G)-A--L-educated T cells after culture *in vitro* with (T, G)-A--L

Cells transferred into irradiated recipients	Antigen (<i>in vivo</i>)	PFC $\pm$ s.e.
B cells*	(T, G)-A--L	200 $\pm$ 39
B cells + normal T cells†	(T, G)-A--L	4,430 $\pm$ 284
B cells + normal T cells + (T, G)-A--L educated T cells after culture <i>in vitro</i> with (T, G)-A--L‡	(T, G)-A--L	576 $\pm$ 166

* 10^7 bone marrow cells.† 10^8 thymocytes.‡ 5×10^6 viable cells.

The direct anti-(T, G)-A--L PFC responses in irradiated Balb/c mice 12 d after receiving transfer of B cells, or B cells and normal T cells, or B cells, normal T cells and suppressor T cells ((T, G)-A--L-educated T cells after culture with (T, G)-A--L *in vitro*), and (T, G)-A--L. Results as geometric means of 15 mice $\pm$ s.e.

Here I describe a method for demonstrating the presence of suppressor T cell activity in a population of primed or 'educated' T cells. Educated T cells, on culture with antigen *in vitro* for a period of 6–8 h, release a specific cooperating factor which can replace T cells *in vivo*¹². The cells remaining in culture at the end of this time have lost the ability to collaborate with bone-marrow-derived (B) cells *in vivo*. Instead, they can suppress the antibody response *in vivo*, as demonstrated by mixing them with normal T cells, B cells and antigen, and transferring into irradiated recipients. Suppression of the immune response by these cells is antigen-dependent, but non-specific in effect. These observations suggest the possible existence of two functional lines of T cells, one cooperative and the other suppressive, and indicates a convenient method for their separation.

The preparation, in Balb/c mice, of T cells educated to the synthetic polypeptide antigen (T,G)-A--L, and the subsequent culture of these cells with antigen *in vitro* has been described in the preceding paper¹². After 6–8 h culture, the cells were spun down and the supernatant removed. The cells were resuspended in cold Eagle's medium, washed twice, and finally resuspended to a concentration of about 5×10^6 viable lymphocytes ml^{-1} . Over the period of *in vitro* culture, a decrease in viability of 15–20% occurred as judged by trypan blue exclusion. The cells were tested for their ability to interact with B cells by transfer together with 10^7 bone marrow cells and (T,G)-A--L (10 μg in saline per mouse) into irradiated (850 rad) syngeneic recipients. In some experiments, described below, 10^8 normal T cells (thymocytes) were also present in the mixture transferred. Twelve days after transfer, the direct anti-(T,G)-A--L plaque-forming cell (PFC) response in the spleens of these animals was measured as previously described¹². The results in the tables are geometric means of 15 mice $\pm$ s.e.

T cells educated to (T,G)-A--L were able to cooperate with B cells *in vivo* (Table 1), provided they had not been previously cultured with antigen *in vitro*. After incubation with (T,G)-A--L *in vitro* for 6–8 h, the educated T cells could not collaborate with B cells *in vivo* in the response to (T,G)-A--L. Moreover, these cells caused the normal background response observed when B cells and (T,G)-A--L are transferred into irradiated hosts to drop to zero; this was the first suggestion that they were endowed with suppressive properties. This dramatic change in the *in vivo* activity of the educated T cell population did not occur if antigen was omitted from the *in vitro* culture system. Furthermore, the change was antigen-specific, since the cells continued to cooperate normally in the response to non-cross-reacting erythrocyte antigens (personal observation).

To test whether the educated T cells had indeed become actively suppressive after incubation with antigen *in vitro*, they were transferred together with B cells, 10^8 normal T

cells and (T,G)-A--L into irradiated hosts. The usually excellent PFC response produced *in vivo* by B cells and normal T cells was reduced by over 90% by the presence of 5×10^6 educated T cells previously cultured *in vitro* with antigen (Table 2).

The specificity of action of these (T,G)-A--L-induced suppressor T cells was examined by transferring them with normal T and B cells and a non-cross-reacting antigen, sheep red blood cells (SRBC), into irradiated hosts. In one group, (T,G)-A--L was also added. The anti-SRBC response was very significantly reduced by the presence of these cells (Table 3), provided the original educating antigen, (T,G)-A--L was also present. If (T,G)-A--L was omitted, however, then the suppressor T cells had no effect on the response to SRBC. Thus the suppressive effect of these cells was apparently non-specific, but the presence of the specific inducing antigen was necessary to elicit it.

It seems that the following events occur when educated T cells are cultured *in vitro* with antigen for 6–8 h. (i) A specific cooperative factor is released which collaborates with B cells *in vivo* in the induction of an antibody response to the educating antigen, as described in the preceding report¹². (ii) The cells lose the normal ability of educated T cells to collaborate with B cells *in vivo* in the response to the educating antigen. (iii) The cells display a potent suppressive influence on B cells and on mixtures of normal T and B cells *in vivo*. In contrast to the specific cooperative activity of educated T cells or the T-cell factor, the suppressive activity is non-specific in its effects, although the original educating antigen must be present for non-specific suppression to occur.

These results suggest the conclusion that a primed T-cell population contains two functionally distinct groups of cells, one of which is specifically cooperative, while the other is non-specifically suppressive. Brief *in vitro* culture stimulates the cooperative T cell population to release its cooperative factors, but also renders it apparently exhausted at the end of 8 h, although this exhaustion might be only temporary. The suppressive T cell population, on the other hand, is not exhausted by this *in vitro* incubation, and its activity is therefore seen to the full when the cells are transferred with B cells *in vivo*. It seems likely that the suppressor T cells release non-specific suppressive substances shortly after stimulation by antigen *in vivo*, as judged by the efficiency with which the response given by normal T and B cells to SRBC was suppressed by (T,G)-A--L-induced suppressor T cells and (T,G)-A--L. It is also probable that the suppressor T cells constitute an important physiological control mechanism *in vivo*, here demonstrated in the antibody response, but most

TABLE 3 Specificity of suppressor T cell activity: ability of (T, G)-A--L-induced suppressor T cells to inhibit the response to SRBC *in vivo*

Cells transferred into irradiated recipients	Antigens (<i>in vivo</i>)	PFC $\pm$ s.e. (anti-SRBC)
B cells*	SRBC	328 $\pm$ 108
B cells + normal T cells†	SRBC	4,860 $\pm$ 411
B cells + normal T cells + (T, G)-A--L-induced suppressor T cells‡	SRBC	5,640 $\pm$ 384
B cells + normal T cells + (T, G)-A--L-induced suppressor T cells	SRBC + (T, G)-A--L	844 $\pm$ 110

* 10^7 bone marrow cells.† 10^8 thymocytes.‡ 5×10^6 viable cells.

The direct anti-SRBC response in irradiated Balb/c mice 10 d after receiving transfer of B cells, or B cells and normal T cells, or B cells, normal T cells and (T, G)-A--L-induced suppressor T cells, and antigen. Suppressor T cells were prepared by incubation of T cells educated to (T, G)-A--L with (T, G)-A--L *in vitro* for 8 h. Results as geometric means of 15 mice $\pm$ s.e.

likely applying to cell mediated immunity also¹³. Other suppressive phenomena, including some forms of antigenic competition^{8,9}, infectious tolerance¹⁰ and the graft-versus-host reaction^{6,7}, may also be mediated by the same T cell sub-population. The system described here provides a method for the functional separation of the cooperative and suppressive T cell lines, and should open the way to further clarification of their properties.

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Cyclic GMP Response *in vivo* to Cholinergic Stimulation of Gastric Mucosa

INTEREST in cyclic guanosine 3',5'-monophosphate (cGMP) has been stimulated by the demonstration that its sister nucleotide, cyclic adenosine 3',5'-monophosphate (cAMP), is an intracellular mediator or 'second messenger' for many hormones, including noradrenaline, the neurohormone of the sympathetic nervous system¹. Reports concerning the possible implication of cAMP in gastric secretion in mammals are conflicting. The infusion of histamine increases cAMP in canine gastric juice² but does not stimulate adenylate cyclase activity of canine gastric mucosa³. A review of the subject has led to no consensus of opinion⁴.

An association of cholinergic stimuli with increased tissue cGMP has been described in several organs. Addition of acetylcholine (ACh) *in vitro* increased cGMP in rat heart and liver, rabbit brain, dog thyroid and guinea pig ileum⁵⁻⁸. Mouse brain showed an increase of cGMP with infusion of a cholinergic agonist but not after pretreatment with atropine⁹. Isolated strips of rat gastric fundus contracted when exposed to cGMP¹⁰.

To investigate the possibility that cGMP might be a mediator for cholinergic nervous stimuli in the stomach, an *in vivo* preparation making possible the direct stimulation of the vagus nerves was devised. Acid secretion was demonstrated by measurement of the pH of the gastric mucosal surface. Strips of gastric mucosa (dissected free of the underlying muscularis) were removed from (barbiturate)-anaesthetised adult mongrel dogs and instantly frozen in liquid nitrogen immediately before and after various experimental manipulations. The separation from the blood supply before completion of freezing never exceeded 5 s. The mucosal samples (1 to 2 g) were extracted with trichloroacetic acid and fractionated on a formate column. Cyclic GMP was assayed by radioimmunoassay^{11,12}. The

specificity of the assay was verified by demonstrating the absence of cross reactivity with cAMP and the major mono-, di-, and tri-phosphate nucleotides. Reproducibility was within 5%. Sensitivity was less than 2 pmol cGMP per g tissue. Known amounts of cGMP added to one of two identical samples were quantitatively recovered. ³H-cGMP was used as a recovery standard for the extraction process. All assays were run in triplicate.

Sham operations produced no change in mucosal cGMP content. Results of the various procedures are presented in Table 1. Electrical stimulation (30 pulses (10 ms, 22 V) s⁻¹ for 1 min) of the distal thoracic vagus nerves cut just above the diaphragm more than doubled mucosal cGMP in the fundus of the stomach but did not change antral mucosal cGMP. Pretreatment with intravenous atropine (0.2 mg kg⁻¹), a cholinergic antagonist, prevented the increase in fundic mucosal cGMP.

Table 1 Cyclic GMP Content of Canine Gastric Mucosa

	Fundus			Antrum		
	(pmol g ⁻¹)	N	P	(pmol g ⁻¹)	N	P
Baseline	28 ± 2			49 ± 4		
Vagal stimulation *	66 ± 9	8	<0.001	45 ± 5	5	<0.2
Baseline after atropine	39 ± 5			49 ± 8		
Vagal stimulation after atropine	35 ± 6	4	>0.3	36 ± 1	2	>0.2
Baseline	58 ± 1			57 ± 3		
ACh infusion *	104 ± 5	4	<0.001	58 ± 6	3	>0.5
Baseline	30 ± 5			40 ± 2		
Insulin infusion *	53 ± 10	4	<0.02	37 ± 6	4	>0.5
Baseline	35 ± 4			42 ± 7		
Insulin infusion, vagi cut	31 ± 4	4	>0.2	37 ± 5	3	>0.1
Baseline	30 ± 2					
Pentagastrin infusion *	29 ± 2	4	>0.5			
Baseline				72 ± 6		
Vagal stimulation, fundus out				69 ± 5	4	>0.5

* Acid secretion (reduced gastric mucosal pH).

Infusion of ACh iodide (0.05 mg in 1 min) into the coeliac artery produced a two-fold increase in fundic mucosal cGMP and no change in the antrum, duplicating the results of vagal stimulation. Cannulation of the coeliac artery for infusion was preceded by dissection of the sympathetic-rich coeliac plexus, which may account for an increase in baseline mucosal cGMP in these experiments. Vagal stimulation achieved by hypoglycaemia after intravenous injection (0.6 units kg⁻¹ in a bolus) of crystalline zinc insulin (blood sugar below 50 mg per 100 ml in all dogs) caused a nearly two-fold increase in fundic cGMP with no change in the antrum. Vagus section eliminated the fundic cGMP response to insulin.

Because changes in fundic mucosal cGMP could result from release of the hormone gastrin from the antrum in response to vagal stimulation, the effect of pentagastrin in concentration sufficient to produce acid secretion (1 µg kg⁻¹ over 6 min, infused intravenously) was tested. There was no effect on the cGMP content of the fundic mucosa. Thus, the increase in fundic cGMP with vagal stimulation does not appear to be mediated by gastrin or by the presence of acid in the stomach but is more likely a direct response to cholinergic stimulation.

It seemed possible that the gastric antrum might not be responding to vagal stimulation with a change in cGMP because of the suppressive effects of fundic acid on gastrin-producing cells in the antrum. Therefore, the fundus was resected in four dogs with vagal innervation of the antrum preserved intact, as shown by antral motility when the vagi were stimulated. After removal of this acid-secreting area, vagal stimulation still failed to alter antral mucosal cGMP. The high baseline cGMP in antral mucosa following fundectomy is unexplained.

These data demonstrate an association between several types of cholinergic stimulation of the stomach *in vivo* and an increase in canine fundic mucosal cGMP and suggest that cGMP may serve as a 'second messenger' for ACh, the 'first messenger' of cholinergic stimuli. Cyclic GMP may not perform this function in the gastric antral mucosa, but it is possible that antral mucosal cholinergic receptors are too few in number to raise cGMP to a level within the sensitivity of this assay. The results with gastric fundic mucosa add to the growing body of evidence linking cholinergic stimulation with increases in tissue cGMP.

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Colchicine and Vinblastine Inhibit Fibroblast Aggregation

THE alkaloids colchicine and vinblastine interfere with the assembly of microtubules, yet they also affect several properties of the surface membrane of animal cells. They disorganise an apparent topographical separation between surface areas involved in membrane transport and in phagocytosis in rabbit polymorphonuclear leukocytes¹, inhibit the agglutination of these cells by concanavalin A (ref. 2) and inhibit the concanavalin A-dependent adherence of red blood cells to transformed 3T3 fibroblasts³.

We have now found that these alkaloids also inhibit the spontaneous aggregation of hamster fibroblasts (BHK 21, clone 13 (ref. 4)) which occurs when these cells are shaken in suspension, after dispersal from monolayers by exposure to trypsin in the presence of EDTA (ref. 5). We used experimental conditions and an assay involving the Coulter counter, with which we have previously shown that aggregation is suppressed at low temperature, reversed by low concentrations of proteolytic enzymes⁵, stimulated by neuraminidase⁶ and much reduced in a number of polyoma-transferred derivatives of BHK21 cells⁷.

The inhibition by the alkaloids (Figs 1 and 2) is less complete than can be obtained by treating the cells with high levels

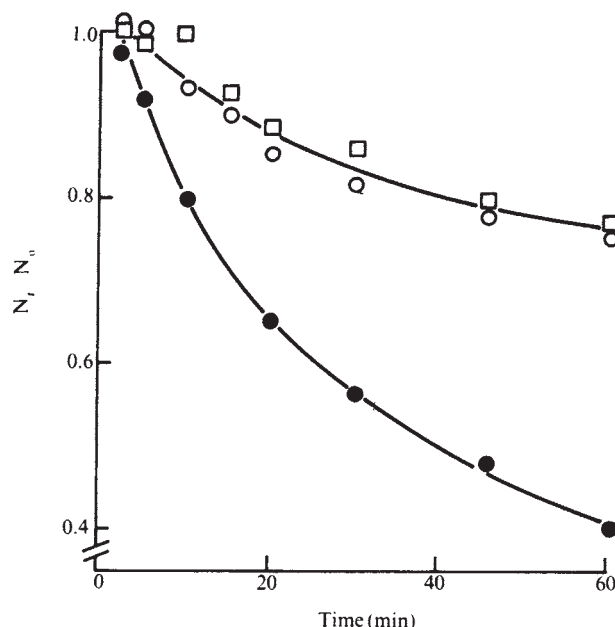


Fig. 1 Inhibition of aggregation of BHK21 cells in suspension by 10^{-4} M colchicine ○ and 10^{-6} M vinblastine □, determined by Coulter counting. These suspensions and control minus alkaloids ● were all aggregated in Hanks' solution buffered with 0.01 M Hepes, pH 7.4. Cells were suspended at an initial density $N_0 = 1.0 \times 10^6$ cells ml^{-1} . Each curve was obtained from 4 ml of suspension incubated in a reciprocating shaker at 37° C in a 10 ml stoppered conical flask. 0.1 ml aliquots were diluted $\times 200$ for counting. The counter measures the total concentration N_t of cells plus cell-clusters.

of trypsin (J. G. E., J. A. Campbell, R. T. R., and M. Vicker, in preparation), and the inhibited cells aggregate more than do polyoma-transformed cells⁷. Vinblastine is effective at much lower concentrations than colchicine, colcemide is intermediate (Fig. 3). Our own unpublished observations show that these concentrations are similar to those required to alter the shape of BHK cells in culture from fibroblast-like to epithelial-like⁸. Lumicolchicine⁹ shows slight inhibition, but only at concentrations 100 times higher than the colchicine from which it was prepared by UV irradiation. The onset of inhibition is fast, occurring within the few minutes resolvable by the assay, and some disaggregation is commonly observed when vinblastine, for example, is added to a partly aggregated cell suspension (Fig. 4). In contrast to these effects on spontaneous aggregation, the agglutination of trypsinised BHK21 cells by concanavalin A seems to be wholly insensitive to the same inhibitors. This is true both for C13 cells (in which case lectin-induced and spontaneous aggregation are superimposed in the assay) and for trypsinised polyoma-transformed BHK21 cells (Fig. 5).

Vinblastine has a very different structure from colchicine, and inhibits aggregation at much lower concentrations. Lumicolchicine, which shares at least some of the non-microtubule effects of colchicine¹⁰, is essentially inactive. It therefore seems more likely that the effects we have observed result from interference with the assembly of microtubules than from some secondary, non-specific effect of the alkaloids on the cell surface. (A remaining problem is that our unpublished observations show that the effect of vinblastine is at least partially reversible, whereas effects of vinblastine on microtubules in some systems are not¹¹.) Microtubules are probably present in trypsinised BHK cells, although Goldman and Follett¹² found them rather sparse and randomly scattered throughout the cytoplasm. It is an intriguing problem how the integrity of microtubules could modify the probability that suspended cells will adhere to one another, and we consider here three possible mechanisms.

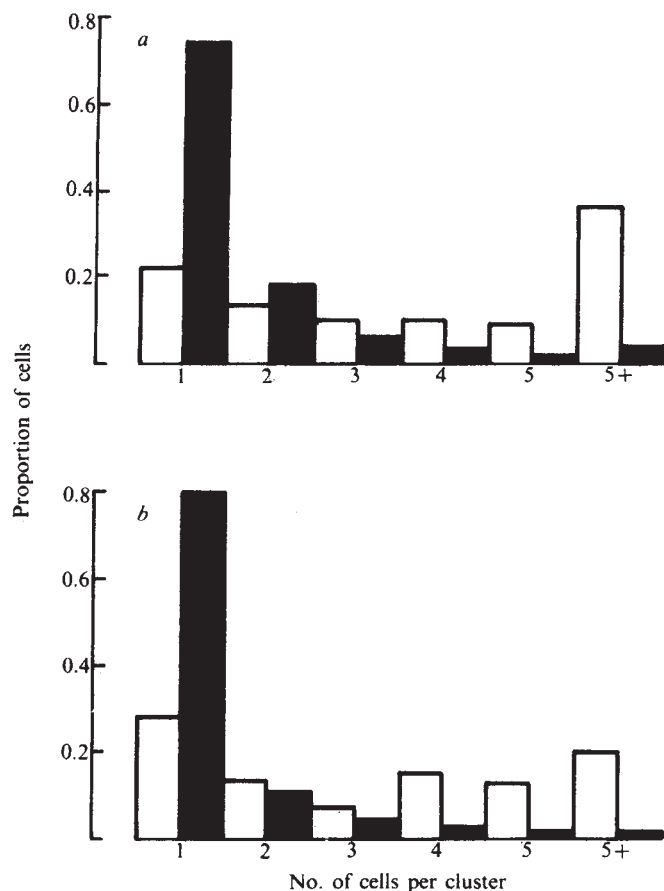


Fig. 2 Inhibition of aggregation by, *a*, 2×10^{-6} M vinblastine and, *b*, 10^{-5} M colchicine, measured by haemocytometry. The ordinate is the fraction of cells found after 45 min incubation in clusters containing the number of cells per cluster shown on the abscissa. Solid figures in the presence of inhibitor, open in absence. About 1,000 cells were counted for each distribution.

First, microtubules may influence the state of convolution of the surface. For example, trypsinised BHK cells are known to possess microvilli¹³ and a relation between fine projections from cell surfaces and cell adhesiveness has often been suggested^{14,15}. The microvilli of BHK cells seem, however, to contain microfilaments rather than microtubules, and are presumably not analogous to the filopodia of mesenchyme cells in the sea-urchin embryo¹⁶, or the axopodia of heliozoans¹⁷. The second mechanism depends on the assumption that the self-adhesiveness of trypsinised BHK cells requires some appropriate arrangement in the surface membrane of 'contact sites' such as those described in *Dictyostelium*¹⁸. We have no evidence for this, beyond a study of the kinetics of aggregation which previously suggested that trypsinised BHK cells behave as though they are adhesive only in patches¹⁹. Berlin has suggested that microtubules can stabilise arrangements of antigenic determinants and other functional elements which would otherwise be capable of rapid lateral diffusion in the membrane¹. This could be achieved by a direct linkage between microtubules and surface elements.

The third mechanism, which we favour at present, is suggested to us by the observations of Vasiliev *et al.* who found that colchicine and vinblastine caused the ruffling activity of fibroblasts locomoting in culture to spread from a restricted area to all parts of the edge of the cells²⁰. Such ruffling has been equated with the insertion, movement and recycling of surface membrane, perhaps by vesicles transported within the cell^{21,22}. Microtubules could be involved in the transport of such surface and perhaps focus its insertion to a restricted part of the cell surface. We propose that cell surface may be

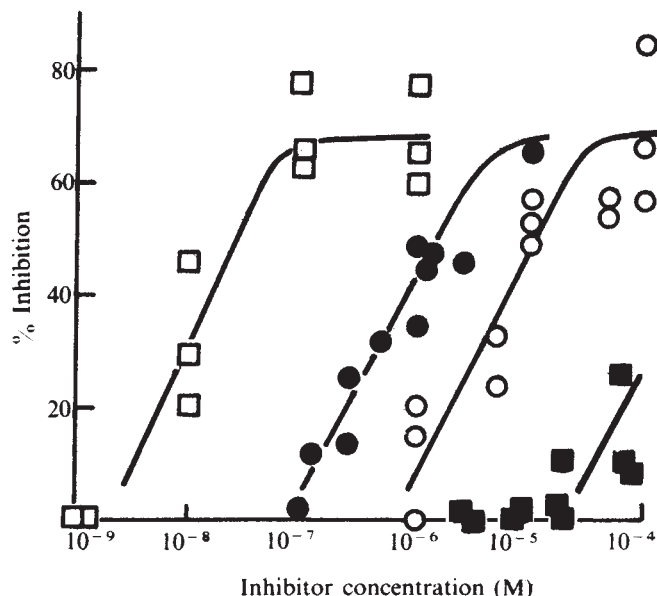


Fig. 3 Concentration dependence of the inhibition of aggregation by vinblastine $\square$, colcemide $\bullet$, colchicine $\circ$, and lumi-colchicine $\blacksquare$. The ordinate is $\frac{N(\text{inhibited}) - N(\text{control})}{N_0 - N(\text{control})} \times 100$, determined after 45 min by Coulter counting as Fig. 1.

adhesive only immediately after undergoing such organised transport, so that aggregation would depend on continued operation of such a cycle. Such a mechanism could account for the sensitivity of aggregation to combined inhibition of glycolysis and respiration which we shall report elsewhere. It is tempting to speculate that a similar surface extrusion phenomenon could be involved in the ADP-dependent aggregation of platelets, which is also sensitive to metabolic inhibitors²³ and to colchicine and vinblastine²⁴.

Whatever the mechanism, if it proves to be true that microtubules can control the distribution of adhesive components in

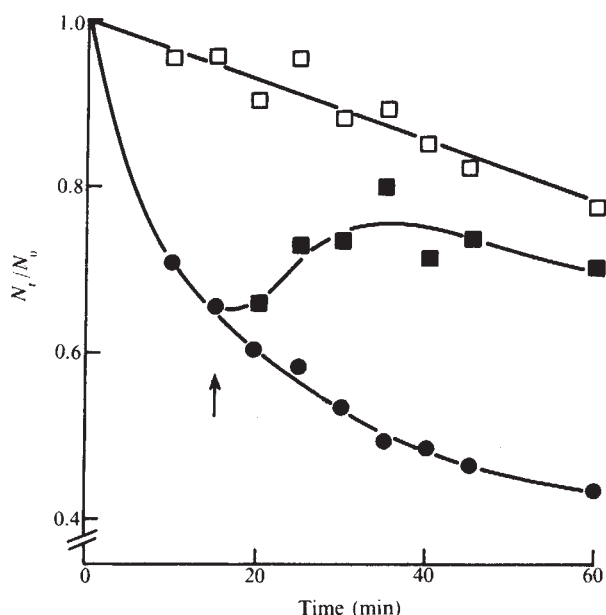


Fig. 4 Kinetics of inhibition of aggregation by 4×10^{-8} M vinblastine, determined by Coulter counting. Vinblastine was added before shaking $\square$ or at 15 min $\blacksquare$. No addition $\bullet$.

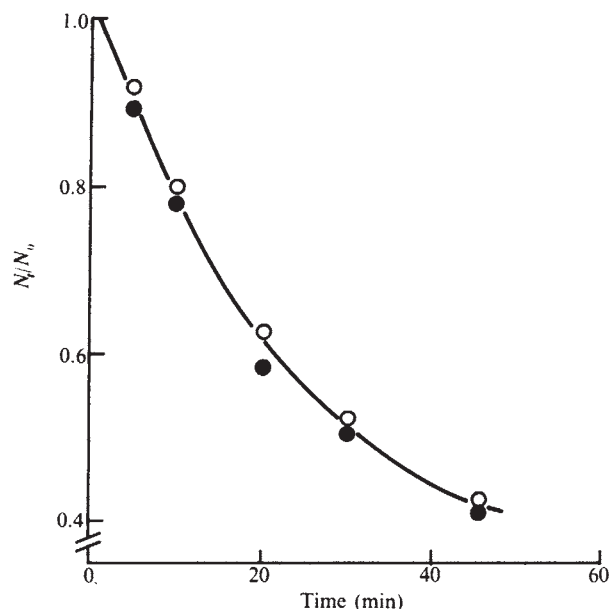


Fig. 5 Lack of inhibition of agglutination of polyoma-transformed cells by concanavalin A ($10 \mu\text{g ml}^{-1}$) by 10^{-4} M colchicine. ○ Con A + colchicine, ● con A only. These cells show a decrease in Coulter count less than 0.1 in the absence of lectin.

cell surfaces, this may be of considerable importance in the organisation of tissues, since it could provide a mechanism whereby cells relate their internal spatial organisation to their orientation with respect to neighbouring cells.

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Explanation of Degree of Correlation of Sibling Generation Times in Animal Cells

SEVERAL methods are available for producing synchronous cultures of mammalian cells^{1,2}, but it is technically impossible to produce a culture which maintains a high degree of synchrony for more than one or two rounds of division because of the highly variable length of the period between successive mitoses from cell to cell. Variability of generation time is also observed in sibling cells formed by the division of a common parent. If cells are able to divide accurately siblings would be expected to be genetically and biochemically identical, and should therefore also have identical generation times but in fact while there is a clear correlation³⁻⁶ it is always imperfect.

In the past this heterogeneity has been implicitly explained in terms of the nature of the sequence of events which a cell must coordinate in order to divide successfully. Because it was considered to be so highly complex, it seemed reasonable to suppose that small differences at each step could accumulate and eventually lead to the great variability in the total intermitotic time observed, even in the case of siblings.

A model of the cell cycle has recently been proposed, however, which positively predicts a high degree of heterogeneity of generation times, but which, in contrast to more conventional models, places much the greatest part of the

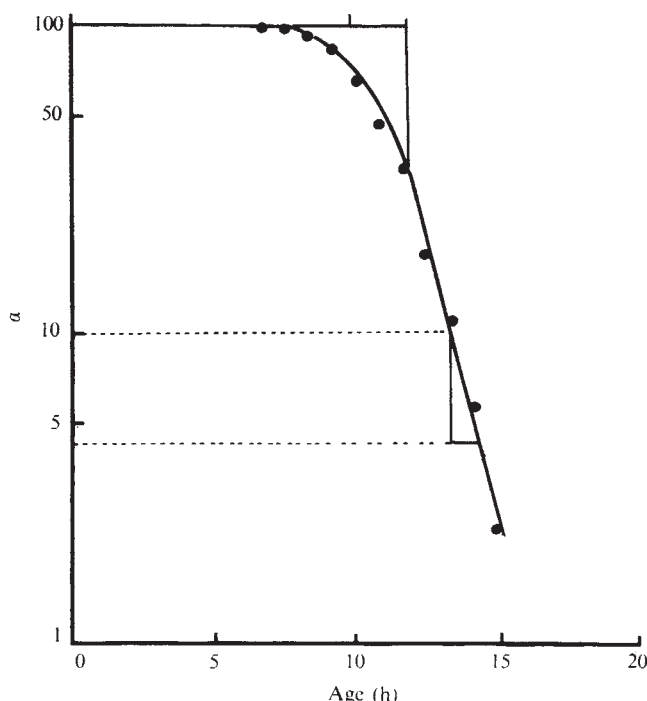


Fig. 1 Semilogarithmic plot of % cells (α) undivided at time t , against t for BHK21/C13 cells. $P = (10 - 4.3)/10 = 0.57 \text{ h}^{-1}$.

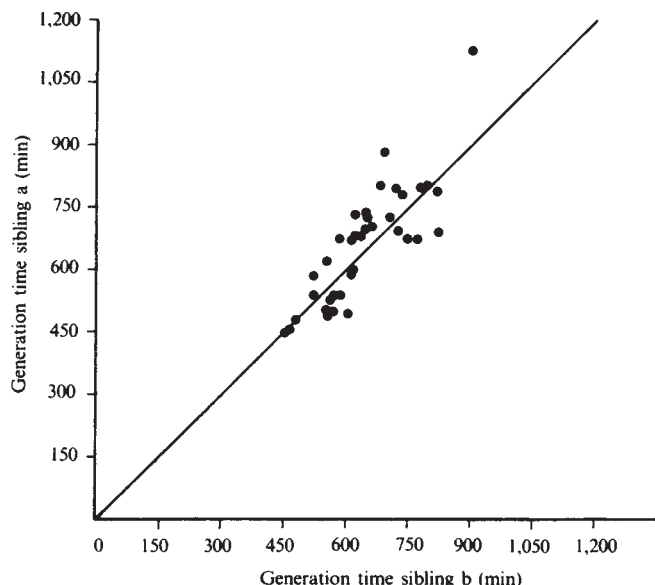


Fig. 2 Correlation between the generation times of siblings for BHK21/C13 cells.

variation at a single point in the cell cycles. According to this theory, proposed in one form by Burns and Tannock⁷, and later by Smith and Martin⁸, there is a sequence of orderly and obligatory events which must be carried out for a successful division. A cell may be either performing this sequence of events, and therefore committed to division (when it is said to be in B phase), or it may be in the alternative condition which Smith and Martin termed the A state.

The A state is regarded as a 'limbo' in which a cell may remain indefinitely without ill effects. A cell in the A state is in no sense committed to division, but is in an unstable state in so far as it may so commit itself at any time, and its probability of doing so is greater the better its physiological condition. A cell in the A state is analogous to a radioactive nucleus in that both may 'decay', the cell into the division sequence of B phase, the radioactive nucleus into its products. Whereas a whole population of cells in the A state will decay at a rate governed by physiological conditions, and a population of radioactive nuclei will decay at a rate governed by the isotopic half-life; it is impossible to state that a specific cell in the A state, or a specific radioactive nucleus is about to decay. The cell's past history (that is, how long it has been in the A state) like that of the radioactive nucleus (how long it has been in existence) does not affect its probability of decaying in the immediate future. Consequently a population of cells in the A state, like a population of radioactive nuclei, will decay with first order kinetics while the time an induced cell spends in the A state is completely unpredictable and therefore by the definition of the word, completely random. It is this random element introduced by the existence of the A state which is said to produce the heterogeneity of intermitotic times in cell populations.

The model makes a number of quantitative predictions which were in good agreement with published observations⁸. The object of this work was to see if it could also provide an adequate explanation of the extent of the correlation of sibling generation times.

BHK21 C13 cells were grown on 30 mm plastic Petri dishes (Nunc, Roskilde) in Dulbecco's modification of Eagles medium supplemented with 10% calf serum. Cells were seeded as sparse cultures the day before filming began, and

gassed with 10% CO₂ in air during incubation at 37° C. Exposures were made at 60 s intervals for 24 h (about two generation times under the conditions used) and the individual intermitotic times measured by frame-by-frame analysis. A cell was said to have entered mitosis when it had completely rounded up before division.

The model predicts a specific type of distribution of intermitotic times. No cell will be able to divide in a time shorter than the length of the necessary sequence of events which makes up B phase (T_b). The remainder of the intermitotic time is entirely attributable to the time spent in the A state, and will therefore have a logarithmic distribution, so that a plot of the fraction of cells (α) having an intermitotic time greater than t against t will show that α remains constant at 100% for a period (which will be equal to T_b) and then decays exponentially just as a population of cells in the A state decays exponentially (because elements of the intermitotic time greater than T_b are spent in the A state). A total of eighty-eight intermitotic times was measured from the BHK cells and is plotted in this form in Fig. 1. The data deviate from the ideal, but for the purpose of discussion this will be assumed to be due to variation in the duration of B phase. On this basis T_b was estimated to be $9.5 \text{ h} \pm 0.6 \text{ h}$ and the transition probability (defined as the fraction of cells leaving the A state h^{-1}) to be 0.57 h^{-1} .

The eighty-eight intermitotic times measured included a total of thirty-seven sibling pairs, whose intermitotic times were well correlated ($r=0.85$, $P \ll 0.001$). There was, however, as expected, noticeable scatter from the perfect correlation line (Fig. 2).

The model proposes a two-part cycle, one being the division sequence, and it will be assumed in what follows that this sequence is of identical duration in siblings, that is, that a cell is able to divide its genetic and biochemical capital with total accuracy between its progeny. The other part of the cycle, however, is of essentially random length, and totally independent of genetic or biochemical endowment for an individual cell, and so unrelated in siblings. On these assumptions, cells of cultures which spend a long period in

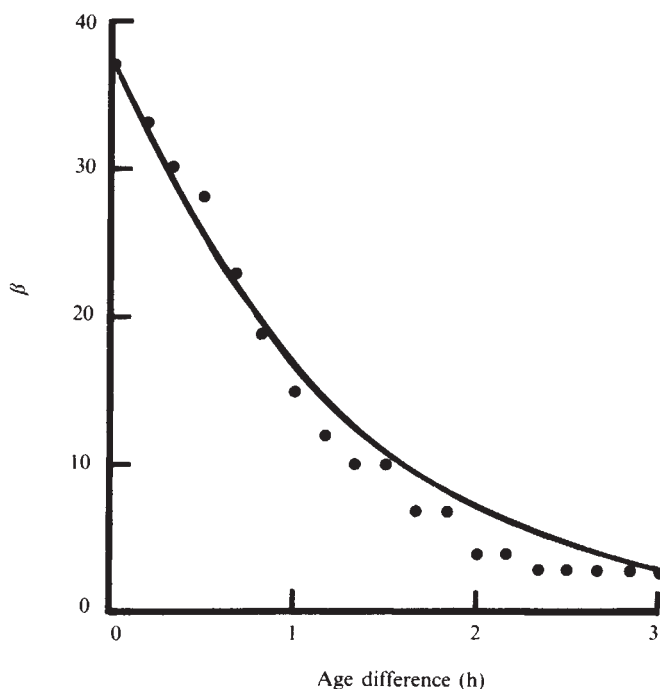


Fig. 3 Semilogarithmic plot of the number of sibling cell pairs (β) with differences in generation time greater than t against t for BHK21. C13 cells, compared with expected line calculated for $P=0.57 \text{ h}^{-1}$, from Fig. 1. $\beta_t = \beta_0 P^t$, where $\beta_t = \beta$ at time t , P =transition probability.

the A state on average will have, on average, a larger random element in their cycle than cells of cultures which do not, and will therefore also have a greater scatter from the perfect correlation line than rapidly growing cells.

In fact it is possible to make a quantitative prediction concerning the distribution of the differences of intermitotic times of siblings on the grounds that as they represent the differences between times spent in the A state, they will have the same distribution as times spent in the A state. Expressed more rigorously, a transition probability of 0.57 h^{-1} (as here) means that a given cell in the A state has a probability of $(1.00-0.57)$ or 0.43 of not leaving the A state in the next hour. If two cells in the A state are considered at the time when the first one leaves, the second will have a probability

directly. The data presented here are consistent with the distribution predicted in this way.

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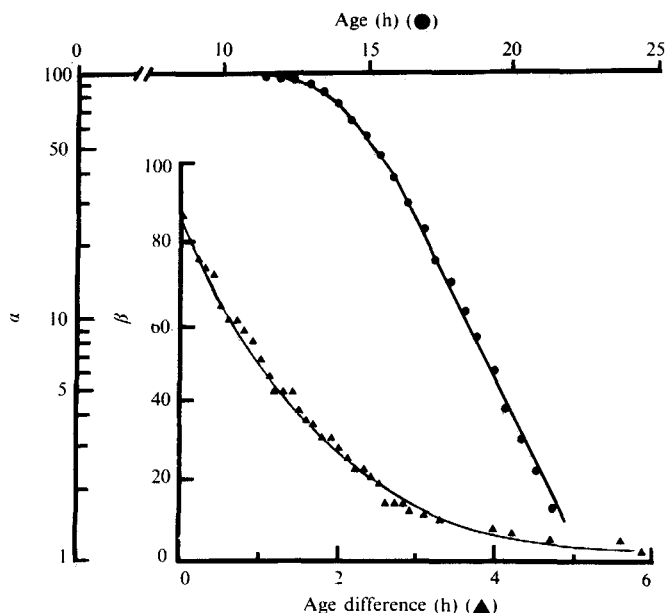


Fig. 4 Semilogarithmic plot of α and β against t for *Euglena gracilis* (data of Cook and Cook³). $P=0.44$.

of 0.43 of remaining in the A state for at least one more hour. Therefore if differences of the intermitotic times of siblings are considered (which have been assumed for the purposes of this treatment to be totally due to differences in the time spent in the A state) a fraction equal to 0.43 of the total will be greater than 1 h . Similar considerations may be applied to different time intervals, and it is consequently possible to deduce a theoretically expected line for the number of sibling pairs (β) whose intermitotic times differ by more than t , against t . Such a line is shown in Fig. 3 for the BHK cells with the actual differences observed. The fit is reasonably good, considering the small numbers involved. Figure 4 shows the plots of α and β for *Euglena gracilis* (data of Cook and Cook³) which involved a far larger number of cells. The agreement between the theoretical and actual distributions is good.

Most current ideas on the cell cycle make no quantitative predictions about the distribution of differences between siblings to be expected, and still less about the relationship between sibling differences and the overall variability of the population. Indeed, there is no *a priori* reason why such a relationship should exist.

In contrast the recently proposed model of the cell cycle^{7,8} does imply such a relationship, in that it should be possible to predict the distribution of the differences between sibling generation times from the distribution of generation times

Autosomal recessive mutation in sugar response of *Drosophila*

THE molecular basis of sugar reception in vertebrates and insects still remains undefined. The fact that stimulation with a solution of a pair of sugars exhibits either synergism or an inhibitory effect^{1,2} suggests that multiple receptor sites are involved. Interactions of certain proteins with given sugars are known both in mammals^{3,4} and in insects^{5,6}, but their relation to sugar reception is not clear. Genetic alteration, which has been successful in studies of chemotaxis in a bacterium⁷ and olfaction in an insect^{8,9}, may throw a new light on such a complex system. For genetic dissection of sugar reception, *Drosophila* offers certain advantages. Methods for measurements of its sugar responses are readily available¹⁰, and procedures to isolate its mutants are well established^{9,11}. We shall describe here the first mutant of sugar response in *Drosophila*.

An isogenic line (AA75-3) of *Drosophila melanogaster*¹⁰ was maintained on the usual sucrose-corn-yeast extract medium. Males treated with the mutagen, ethylmethanesulphonate (EMS)⁹, were mated singly for 4 d with untreated virgin females of the marker strain T5 having genotype *Cy/Pm*; *Ubx/Sb* (*Cy*, *Curly* wings with inversions; *Pm*, *Plum* eye colour; *Ubx*, *Ultrabithorax* halteres with inversions; *Sb*, *Stubble* bristles) with an attached X chromosome, and transferred to be mated with new females of the marker strain T5 for another 4 d. Each F_1 progeny male of *Cy/+*; *Ubx/+* was then backcrossed to three virgins of the marker strain T5. Thus homozygotes, the autosomes of which came from one side of the chromosomal pair of each treated male, were isolated in descendants. From about 1,200 F_1 males, only 100 isogenic lines were obtained (about 300 lines possessing autosomal recessive lethal genes have been maintained with *Cy* or *Ubx*, and others were lost due to the lethal effects of the mutagen).

To detect suspect lines revealing abnormal behaviour, we examined changes in sugar responses of about 100 isogenic lines of flies aged 0-3 d with the preference-aversion test according to Isono *et al.*¹⁰, using a balanced system with the following four stimulants placed in four small glass rings in a Petri dish: distilled water, $5 \times 10^{-2} \text{ M}$ D-fructose, $7 \times 10^{-2} \text{ M}$ D-glucose and $2 \times 10^{-2} \text{ M}$ sucrose each in a 2% agar solution. Concentrations of sugars were determined so as to attract the parent flies (parent strain AA75-3) at a ratio of about 1:1:1:0.2 for fructose, glucose, sucrose and water, respectively. The unbalance of the ratio resulting from changes in responses of flies to any of these three

TABLE 1 Responses of mutant, parent, F₁ and F₂ hybrid flies to sugars

Sugars	Concentration (M)	Number of impulses/0.2 sq*			
		Parent	Mutant	F ₁ hybrid†	F ₂ hybrid‡
Sucrose	10 ⁻²	15.7	13.2§	15.5	14.7
	10 ⁻¹	24.6	26.0	25.6	25.9
	10 ⁻²	19.9	17.8§	19.7	19.7
Maltose	10 ⁻¹	25.2	24.5	25.0	25.4
	10 ⁻²	6.7	6.1	6.6	6.7
	10 ⁻¹	15.7	15.0	14.9	15.9
D-Fructose	10 ⁻²	10.6	3.4¶	9.6	6.4
	10 ⁻¹	19.6	14.5¶	19.4	17.5

* Average number of impulses originating in sugar receptors of labellar single chemosensory hairs of *Drosophila* flies aged 0–2 d during 0.2–0.4 s after initiation of stimulations by the 'tip recording method'. Number of stimulations was about 2,000 with 267 hairs of 89 flies (45 ♂, 44 ♀).

† Coming from two reciprocal classes of single crossings of mutant with parent.

‡ Coming from two reciprocal classes of single crossings of F₁ hybrid with mutant.

§ ¶ Significantly different from parent (§, $P < 0.05$; ¶, $P < 0.01$).

|| Significantly different both from parent and mutant ($P < 0.05$).

sugars could be seen at once. Of about 100 lines examined, one line of flies (126B04) showed a marked decrease in response to glucose, giving an average ratio of about 1.2:0.3:1.1:0.2 for fructose, glucose, sucrose and water, respectively.

Such behavioural changes seem to be a consequence of functional modifications in the chemosensory hairs. To define the regions where modifications were involved, nerve responses of labellar chemosensory hairs of the mutant flies (line 126B04) were compared with those of the parent by the 'tip recording method' used in studies of contact chemoreception in dipterous insects^{10,12}. The nerve responses of flies aged 0–2 d were recorded from the tips of three particular type-L labellar single hairs with a glass capillary filled with sugar dissolved in 10⁻⁴ M NaCl solution, which served as a stimulator and recording electrode, and an indifferent electrode inserted into the back of the head¹⁰. Experiments were done at 20° C in a relative humidity range from 60 to 70.

Column 3 and 4 of Table 1 show the average number of impulses originating in the sugar receptors of single hairs of both mutant and parent flies during 0.2–0.4 s after the initiation of the stimulations by 10⁻¹ and 10⁻² M sucrose, maltose, glucose and fructose. The responses of the mutant flies to glucose are drastically different from those of the parent, the average difference being about 5.1 and 7.2 impulses for 10⁻¹ and 10⁻² M glucose, respectively; whereas, with both 10⁻¹ and 10⁻² M fructose, the mutant flies reveal phenotypes similar to those of the parent. The changes of responses in the mutant flies to both sucrose and maltose seem to be dependent on the mutation present; although the mutant flies responded normally to 10⁻¹ M of those sugars, they revealed reductions of about 2.5 and 2.1 impulses for 10⁻² M of sucrose and maltose, respectively. In addition to these four sugars, the labellar hairs of the mutant flies were completely insensitive to 1 M D-galactose, D-mannose and D-xylose, and 3 × 10⁻¹ M lactose as expected from the results with the parent flies¹⁰. Male flies revealed phenotypes similar to those of females of the same strain in response to stimulations by sucrose, maltose, glucose and fructose (for example, 14.6 and 14.4 impulses, to 10⁻¹ glucose, and 3.7 and 3.1 impulses to 10⁻² M for males and females of the mutant flies, respectively). This indicates that the mutation present can be independent of the sex chromosomes, and probably is in the autosomes.

Column 5 of Table 1 shows the average magnitudes of nerve responses of the 20 F₁ hybrid flies, which came from two reciprocal classes of several single crossings of the mutant flies with the parent. It is evident from this table that the F₁ hybrid flies reveal phenotypes similar to those of the parent in response to the four sugars. No significant

difference in response was recognised between sexes. From the above results, we conclude that the mutation is autosomal recessive.

Column 6 of Table 1 shows the average values of impulses of the 40 F₂ hybrid flies, coming from two reciprocal classes of several single crossings of the F₁ hybrid flies with the mutant. The F₂ hybrid flies revealed phenotypes similar to those of the parent in response to sucrose, maltose and fructose; whereas to both 10⁻¹ and 10⁻² M glucose, the F₂ flies gave responses intermediate to those of the parent and mutant strain. Male F₂ hybrids showed similar magnitudes of response to their females. An attempt to define segregations of the F₂ hybrids between phenotypes of both strains on the basis of their responses to stimulation by 10⁻¹ and 10⁻² M glucose has been unsuccessful because of marked variations within each group of the parent and mutant flies, which deterred us from drawing an arbitrary line within the F₂ hybrid flies.

Although the mutation could not be defined other than that it is an autosomal recessive, the fact that the mutant flies revealed simultaneous modifications in response to glucose, sucrose and maltose, though they showed normal responses to fructose, implies that at least two different receptor sites are involved in labellar chemosensory hairs of *Drosophila*. If further genetical analysis shows that the mutation is governed by a single gene, comparisons of the mutant flies with the parent will lead to isolation and identification of receptor sites in the labellar chemosensory hairs.

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Blood Glucose and Nutritive Antibiotic Activity in Ruminants

Numerous monogastric species respond to the continuous ingestion of small amounts of certain antibiotics by increased growth and improved feed utilisation. The mode of action of this so-called "nutritive effect" is not well understood, although various alterations in the intestinal flora have been observed as well as changes in the intestinal mucosa, indicating improved nutrient absorption.

Ruminants also exhibit growth responses to some antibiotics, but here the mode of action is even less clear. Changes in the rumen metabolites and flora are likely to occur, and hypotheses trying to explain the antibiotic growth response in ruminants are largely based on altered rumen fermentation¹. No consistent changes in rumen or host metabolism have so far been demonstrated, however, which could be related to growth responses.

Leskova and Zucker² have recently reported that the growth response of early weaned calves to the new phospholipid-antibiotic Diurmycin (SFI 80.586) was accompanied by elevated blood glucose levels. This antibiotic has a predominantly Gram-positive spectrum and is not active against protozoa. It is not absorbed and therefore its primary action should be confined entirely to the lumen of the intestinal tract. Antibiotic levels of 5 and 10 p.p.m. in the feed were employed, the lower level corresponding to approximately 0.1 mg, or less, per kg of body weight. The calves were fed a complete pelleted feed (maize 40%, oats 15%, alfalfa meal 17%, soya bean meal 25%, minerals and vitamins 3%) with hay.

The results of two experiments are shown in Figs 1 and 2. Similar increases of weight gains and blood glucose levels have been measured in two additional experiments. There were no changes in serum lactate levels. Serum urea concentration decreased significantly in calves receiving the antibiotic, indicating increased protein synthesis³. Diurmycin improved feed utilisation in all experiments by 5 to 10%.

Bergman⁴ showed that blood glucose concentration in sheep is closely related to turnover rate of glucose. This implies that the rate of glucose utilisation is dependent on sufficiently high blood glucose levels. It can be assumed that such elevated blood glucose levels also stimulate insulin secretion⁵ and thereby amino acid transfer into cells. These findings would offer an explanation for the growth response to antibiotics in ruminants, which seems, according to general experience, more pronounced when diets with high roughage content are fed. Such diets tend to lead to a relative "glucose deficiency" not

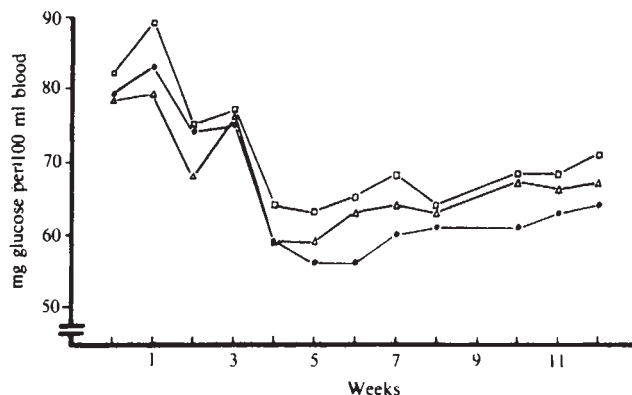


Fig. 2 Relationship between the level of Diurmycin in the feed, growth and blood glucose. Sixteen female, individually fed calves (starting weight 61 kg) were subjected to each treatment. Concentrates were fed *ad libitum* but hay was restricted. With no Diurmycin in the feed (●), the average daily gain in body-weight was 680 g; with 5 p.p.m. (Δ), Diurmycin in the feed the gain was 748 g and with 10 p.p.m. (□) in the feed the gain was 743 g. The arrow indicates the withdrawal of milk replacer.

only by supplying less preformed glucose, but also by favouring formation of acetic acid rather than propionic acid, which is the main precursor of glucose in ruminants.

We conclude that nutritive antibiotic action in the ruminant is related to elevated blood glucose, which may result from either increased rumen synthesis of propionic acid or decreased degradation of starch and sugars in the rumen.

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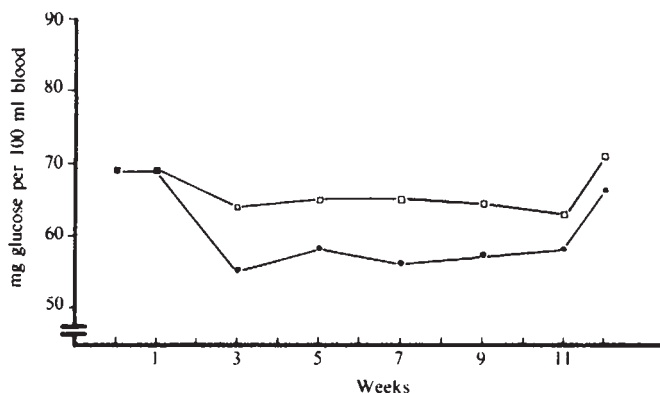


Fig. 1 Effect of Diurmycin on growth and blood glucose. Twelve female, individually fed calves (starting weight 68 kg) were subjected to each treatment. Concentrates and hay were given *ad libitum*. With no Diurmycin in the feed (●), the average daily gain in body weight was 709 g. With 10 p.p.m. (□) of Diurmycin in the feed the average daily gain was 823 g. The arrow indicates the withdrawal of milk replacer.

Structural Difference between Luminal and Lateral Plasmalemma in Pancreatic Acinar Cells

THE acinar cells of the pancreas, as well as other exocrine protein secreting cells, are highly polarised. Release of secretion products, which occurs by exocytosis (fusion of the secretory granule membrane with the plasmalemma (PM) followed by opening at the point of fusion¹, is limited to the restricted portion of the cell surface facing the secretory lumen. It is still unclear whether this selective localisation of discharge depends on the specific properties of the luminal portion of the PM. Studies carried out in other cell systems have indeed revealed that regions of the PM where specialised functions are located can exhibit distinct characteristics²⁻⁵. But in the pancreas a comparative analysis of the secretory portion of the PM relative to the non-secretory portion has never been reported. By the use of freeze-fracture we have now observed that the luminal PM is different in structure from the remainder of the PM. Rather it is reminiscent of the membrane with which it is functionally associated, the membrane of the zymogen granule (ZG).

Male albino guinea pigs were starved for 24 h and then killed by a blow on the head. Small pancreas tissue fragments were fixed for 30 min in 1.5% glutaraldehyde and 1% formaldehyde, freshly prepared from paraformaldehyde, in phosphate buffer, pH 7.3, then infiltrated for 3 h in 30% glycerol in 120 mM phosphate buffer, pH 7.3. Samples were frozen by immersion in Freon 22, cooled to -150°C in liquid nitrogen, then freeze-fractured according to the method of Moor and Mühlethaler⁶ in a Balzers freeze-etching device. Fracturing temperature was -115°C . Platinum-carbon replicas were washed in Na hypochlorite to remove organic material, then in distilled water and recovered on 200 mesh copper grids. The replicas were examined in a Philips EM 200 electron microscope.

Large areas of both the luminal and lateral PM were exposed by cleavage. Sheets of apical PM are easily identified by the presence of a surrounding continuous network of thread-like structures which, from previous studies, are known to correspond to tight junctions⁷ and by the occurrence of rounded elevations or depressions and finger-shaped structures which are identified as cross-sectioned and tangentially exposed microvilli.

It is well known that the PM is a highly asymmetrical structure⁸. In general, after cleavage, most of the particles are associated with the half of the membrane left frozen to the cytoplasm (the A face). The face of the half membrane left frozen to the extracellular space (the B face) bears fewer particles per unit area. In pancreatic acinar cells the typical A face pattern can be found only in the basal and lateral portions of the PM (including the regions close to the tight junctions and within the chambers delimited by their network). But over the entire luminal area there are far fewer particles per unit area of the A face (Fig. 1). The transition is very sharp across the inner continuous line of the tight junction delimiting the lumen, as can be seen when the fracture plane runs continuously from the lateral regions through the junctional complex to the luminal area (Fig. 1a). On the B face the number of particles is always very small, with no clear difference between the luminal and the lateral portions of the PM (Fig. 1b).

The limiting membranes of the ZG stored within the cells bear many fewer particles per unit area than the membranes of the other cytoplasmic organelles. The distribution is again asymmetrical, with more particles in the half membrane left frozen to the surrounding cytoplasm (A face) (Fig. 1, inserts). Hence, if we look at the particle patterns, the B face of the ZG

membrane resembles the B face of the whole PM while the A face of the ZG membrane looks like the luminal PM A face. Since during exocytosis the A leaflet of the ZG membrane becomes continuous with the A leaflet of the luminal PM and the same occurs with the two B leaflets, we have been led to postulate that the morphological similarity is more than a casual event and might be the expression of a similar biochemical composition. Indeed, our previous studies on isolated cellular membranes of the guinea pig pancreas have shown that the ZG membrane and the PM are similar in lipid composition⁹. Also, at least some of the polypeptides present in the ZG membranes seem to be present also in the PM preparations, even if the polypeptide composition of the latter fraction is much more complex¹⁰. Our PM preparations, however, represent portions of the entire surface, whereas a meaningful comparison should be made with the luminal portion only, which accounts for a small proportion of the total fraction¹¹.

It might be argued that the peculiar structure of the luminal PM could only depend on the continuous insertion of ZG membrane patches. Since ZG membranes contain few particles such insertion could result in a 'dilution' of the typical PM particle density. But if this were the case, one would expect to find, at least in some cells, an uneven distribution of particles over the luminal A face (ranging from areas similar to ZG membranes to areas of the typical PM type) and no sharp transition at the level of the tight junction. Our results were exactly opposite to this. Furthermore, in our experiments the animals were starved and in these conditions the rate of discharge of ZG is known to be very low. We believe therefore that the peculiar structure that we have observed is probably intrinsic to the luminal PM and could be maintained by a selective restriction in membrane mixing occurring in the PM at the level of the tight junction.

Recent turnover experiments in which ZG membrane proteins have found to have much longer half lives relative to the secretory proteins are consistent with the idea that ZG membranes can be reutilised in many secretory cycles¹². Thus, once fused with the luminal PM, they should be somehow reinternalised, either as discrete macromolecules or as intact membrane patches, to be reassembled within the cell. In agreement with this conclusion it has been previously observed that a population of vesicles, possibly retrieved from the luminal PM, appears in pancreatic acinar cells following stimulation of secretion¹³. These results, together with those we report, make it tempting to suggest that the ZG membranes and the luminal portion of the PM might be part of a unique pool of membranes which are continuously shuttled backward and forward between the newly formed ZG and the luminal surface. It is interesting that also in other cellular systems (the toad bladder epithelium and the endothelia^{5,14}) the vesicles known to be able to specific interaction (fusion-fission) with the PM have particle pattern and polarity similar to the portion of PM with which they interact. It therefore seems possible that the phenomena which are at the basis of our observations might not be limited to secretory cells but have a more general occurrence.

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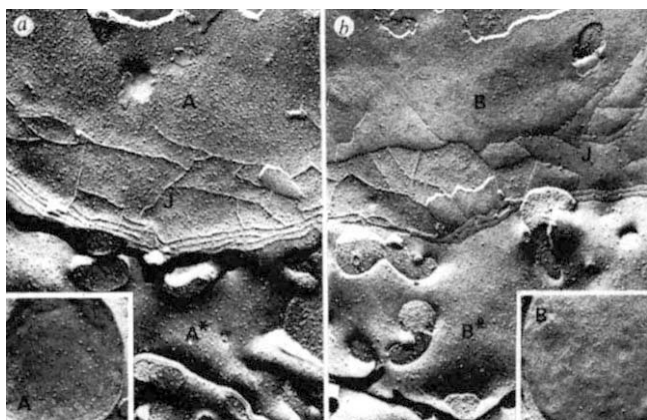


Fig. 1 Freeze-fracture of guinea pig pancreatic acinar cells. The fields illustrate the appearance of large sheets of PM in the regions of transition between the lateral and the luminal (*) portions, and include the tight junctions (J). The face frozen to the cytoplasm is labelled A, that frozen to the extracellular space is labelled B. The inserts illustrate the outer (A) and inner (B) leaflets of the ZG membranes. Shadowing was from the bottom. ($\times 45,000$.)

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Table 2 Results of Transplantation of Human Foetal Tissue to *Nude* Mice

Inoculated tissue	Observation (d)	Fate of graft (accepted/total)
Thymus	19-59	7/13
Lung	13-64	10/12
Myocardium	54-60	0/2
Pancreas	64	1/1
Adrenal	17-39	3/12
Kidney	18-36	2/4
Spleen	21-48	0/7
Liver	17-57	0/10
Testis	23-57	3/5
Ovary	5-33	2/4
Spinal cord	21-57	0/2

Macroscopically, lung, thymus, testis and ovary transplants progressively increased in size. Lung transplant growth was most marked, maximally to $6 \times 8 \times 9$ mm. At autopsy the accepted transplants were well defined, apparently encapsulated in the subcutaneous space, and richly vascularised by regional vessels. When transplants did not take, only sparse yellowish tissue remnants were found. Microscopic examination of accepted organ transplants showed these to be richly supplied by host vessels. There was only a minimal inflammatory reaction consisting of a few mononuclear cells. The histological appearance of these organs was as follows.

The thymus was of normal lobular appearance with some differentiation of cortex and medulla. The epithelial elements in the medulla were further differentiated so that number of Hassall's corpuscles were readily identifiable. The cortical vessels were sheathed by epithelial cells, and the cortex otherwise dominated by lymphocytes with a moderate number of mitotic figures. Reticulum cells and histiocytes were in normal concentrations. Compared with the donor material, the distinction between cortex and medulla was less apparent. An increase in the number of Hassall's corpuscles was noted, but these were of normal appearance (Fig. 1).

The lung showed a domination of markedly dilated alveolar-like structures with a much flattened epithelium. Ducts, the primitive bronchial structures, were clearly seen passing from the alveolar formations. These were lined by one layer of ciliated columnar cells with nuclei placed towards the lumina. Smooth muscle and isolated immature cartilagenous tissue in a mesenchymal stroma were seen in the duct wall together with normal vessels. By comparison with original transplants there was a very marked reduction in mesenchymal tissue elements. (Fig. 2a-c).

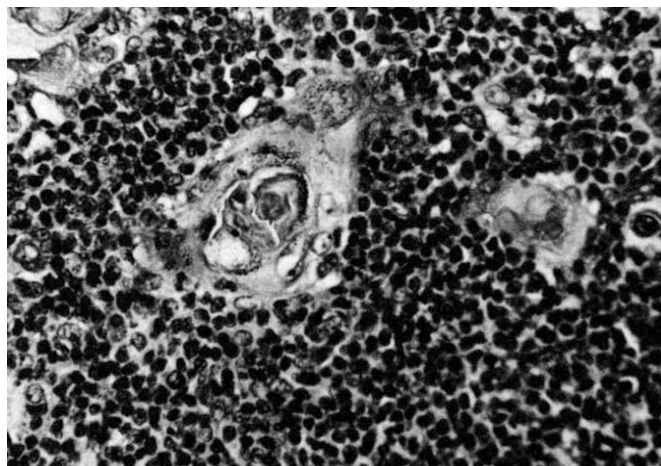


Fig. 1 Human foetal thymus 59 d after transplantation to nude mouse. Hassall's corpuscle surrounded by lymphocytes, histiocytes and reticulum cells $\times 270$. Haematoxylin and eosin (HE).

Heterotransplantation of human foetal organs to the mouse mutant *nude*

THE mouse mutant *nude* displays thymic aplasia which entails a defect in cell-mediated immune responses. This immune deficiency has been demonstrated by transplantation of normal allogeneic skin and heterogeneic skin from mammalian donors including man, and from avian and reptilian donors¹. The *nude* mutant also accepts transplants of many human malignant tumours, including adenocarcinoma of the colon and rectum, malignant melanoma, squamous cell carcinoma and Burkitt's lymphoma (refs 2-4 and C. O. P., unpublished results). We report here the results of experiments involving transplantation of human foetal organs to the *nude* mouse.

Donors were five 14- to 22-week-old human fetuses, the results of pregnancies terminated by Caesarian *sectio parva*. The reasons for termination are given in Table 1 together with the gestational age and sex. All fetuses were considered normal. Immediately after the section, foetal thymus, lung, myocardium, pancreas, adrenal, kidney, spleen, liver, testis, ovary and spinal cord were transplanted in *nude* mice. The inocula measured about $2 \times 1.5 \times 1.5$ mm and were implanted subcutaneously in the lateral abdominal wall.

Table 1 Reasons for Termination of Pregnancies, Gestational Age and Sex of Human Donor Fetuses

Experiment No.	Indications for Caesarian <i>sectio parva</i>	Gestational age (week)	Sex
1	Social	16	m
2	Neurosis neurasteniformis	14	m
3	Rubella	20	f
4	Social	18	m
5	Rubella	22	f

Recipient *nudes* were the 6-week-old progeny of both sexes of the sixth and seventh cycle of backcross mating with Balb/c mice. Breeding was conducted at the Pathological Anatomical Institute, Kommunehospitalet, Copenhagen¹. Testicular and ovarian transplants were restricted to the corresponding sex, but otherwise no account was taken with sex. Animals were observed daily and transplant growth was noted until death or killing.

Tissues were taken from the implantation sites for histological studies. All were fixed in neutral formalin (apart from testis and ovary tissue where Cleland's fixative was used) embedded in paraffin wax, and 7 μ m sections were cut and stained with haematoxylin and eosin and van Gieson-Hansen. Four μ m serial sections of testis and ovary transplants were stained with iron haematoxylin using Rowley and Heller's method⁵. Observation times after transplantation and the results are given in Table 2.

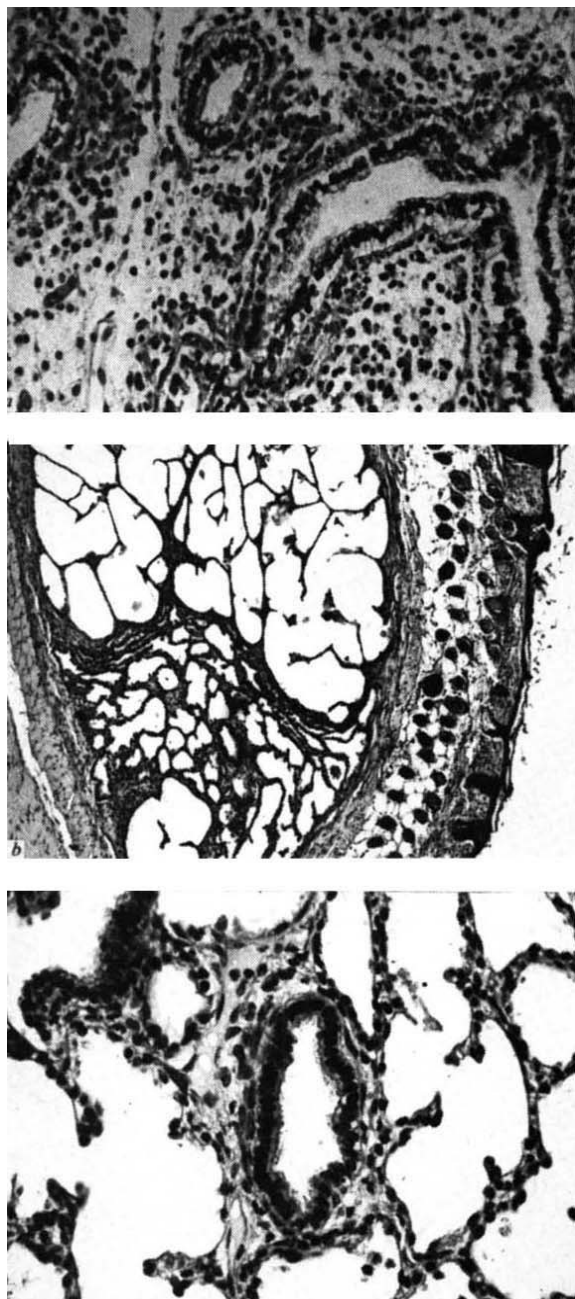


Fig. 2 *a*, Human foetal lung tissue before transplantation (experiment 1). Primitive bronchial structures (ducts) in abundant mesenchymal stroma $\times 270$. *b*, Low power view of same lung tissue 36 d after inoculation in *nude* mouse. The transplant is situated in the subcutaneous space between skin (top) and abdominal muscle layer (bottom). $\times 40$. (HE). *c*, Detail of *b* showing duct surrounded by alveolar-like structures. Note marked reduction in mesenchymal stroma. $\times 242$. (HE).

The pancreas was of normal lobular appearance. Normal acini and small ducts constituted the exocrine elements of the gland, and the islets of Langerhans were normally formed. The histological picture was in good accordance with donor material. In the adrenal, viable tissue comprised only cells of the adult or provisional zone. These were small with round chromatin-rich nuclei which filled most of the cell. The sparse cytoplasm contained few vacuoles. In the foetal zone there was extensive necrosis. No medullary tissue was seen (Fig. 3).

The recovered kidney tissue closely resembled the original transplant. Glomeruli were of variable size, but probably normal in number. Two or three vessel roots were seen with patent lumina and containing erythrocytes. The proportion of

mesangial tissue seemed to be as in the original implant. The visceral and parietal aspects of the Bowman capsules were covered with regular cylindrical epithelium. The capsular spaces seemed to be empty. Tubules were well preserved with an epithelium resembling that of the implant. The proportion of interstitial tissue was increased. This tissue was a loose, connective tissue with many vessels of normal appearance. (Fig. 4 *a, b*).

The general structure of the testis implant was preserved with interstitial tissue containing seminiferous tubules of normal size. The latter contained gonocytes and undifferentiated Sertoli cells. These cells could not be distinguished from normal foetal cells. Some of the interstitial cells resembled fibroblasts while others had some similarity to Leydig cells (Fig. 5).

The stroma of implanted ovaries was of normal appearance and contained a mixed population of oogonia and oocytes. No definite progression in the ovarian development could be demonstrated in the transplants when compared with histology of the tissue before implantation.

At the sites of spleen, liver, myocardium and nervous tissue implantation only necrotic tissue remnants were found with accumulations of macrophages.

Thus the study showed that many human foetal organs (thymus, lung, pancreas, adrenal, kidney, testis and ovary) can be successfully transplanted to the *nude* mouse. Histologically the structure of these organs was essentially preserved, and some development occurred. The appearance of the lung transplants needs a special comment. The dilated alveolar-like structures may be explained by secretion retention dependent on the ectopic lie and by a normal development and differentiation to alveolar-like structures with reduction of mesenchymal elements. This investigation was too small to give any explanation of why some transplants take and others do not, or have not so far.

Other *in vivo* models have been used for human organ transplantation: for example, the chorio-allantoic membrane in the fertilised chicken egg⁶, neonatal rodents⁷ and immunologically tolerant⁸ or immunosuppressed animals⁹. The study organ has generally been human skin, and takes have been achieved for varying periods.

This communication reports the first successful transplantation of human foetal organs to the *nude* mouse. The system seems to offer rich opportunities for the study of growth and differentiation of these organs. Of immediate further interest would be a study of the effects of hormones and carcinogens, as well as toxic, teratogenic, and infectious agents on foetal tissues thus transplanted. The special opportunities which heterotransplanted testicular and ovarian tissue offer will be discussed elsewhere¹⁰. Two important limiting factors need

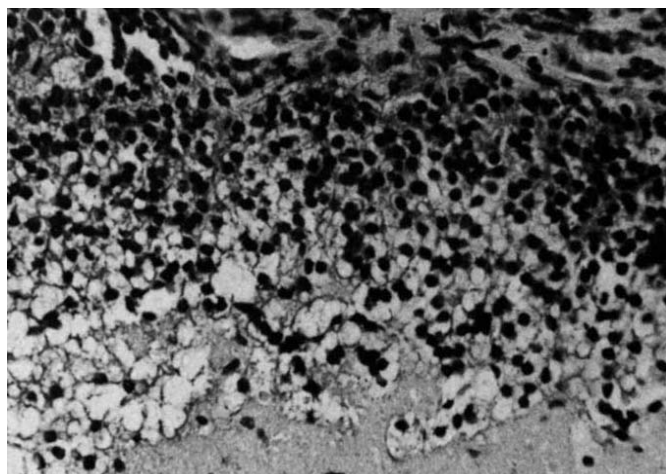


Fig. 3 Human adrenal tissue 28 d after inoculation. Cells from the provisional zone are viable, whereas the foetal zone is necrotic. $\times 270$ (HE).

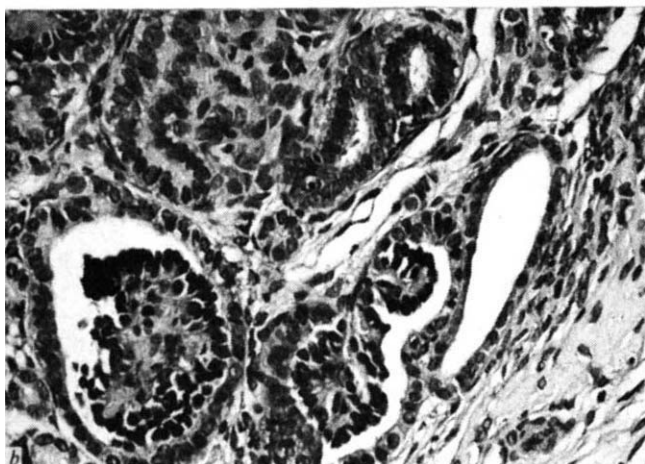
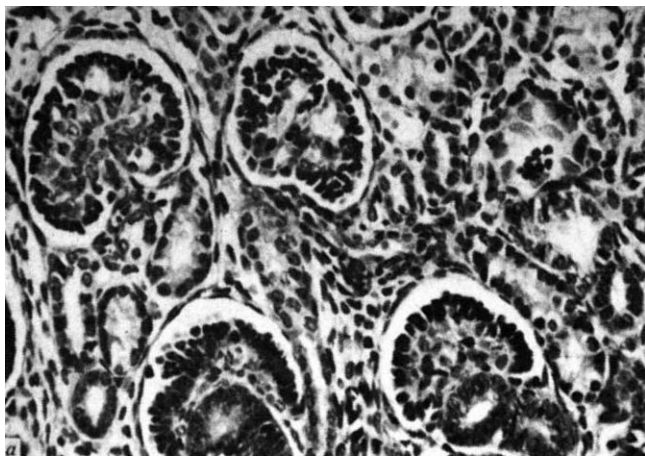


Fig. 4 *a*, Human foetal kidney (exp. 4) before inoculation. $\times 270$ (HE). *b*, Same tissue 36 d after inoculation in *nude* mouse. The primitive glomeruli and tubules closely resemble the donor material $\times 270$ (HE).

however to be mentioned. Donor material is not readily available, and the *nude* mouse is extremely prone to infection which even under good conventional conditions limits the *nude* lifespan to 4 or 5 months. We expect that exploitation of strict specific pathogen free or germ-free milieu will largely compensate for the latter difficulty¹.

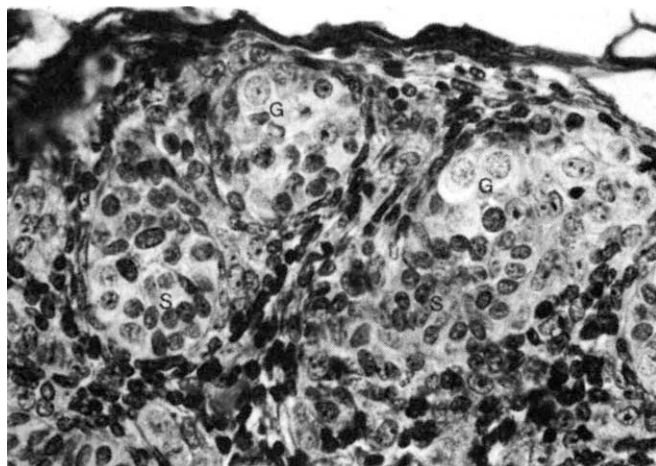


Fig. 5 Human testicular tissue 36 d after implantation. Three seminiferous tubules containing Sertoli cells (S) and gonocytes (G) are shown $\times 340$, iron haematoxylin.

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Efferent control of cricket giant fibres

THE central nervous system not only receives sensory input, but also controls the nature of that input. Efferent control of sensory pathways was suggested on the basis of behavioural experiments by von Holst and Mittelstaedt¹ and by Sperry², who called the postulated mechanism efference copy and corollary discharge, respectively. They argued the need for an efferent system to compensate for the stimulation received during behaviour, and which would otherwise be interpreted as due to events occurring in the environment.

An early example of efferent control was the gamma system of vertebrate muscle spindles³, and since then several examples of control of sensory pathways at various points, from the primary receptors to deep within the central nervous system, have been described^{4,5}. But it has been difficult to show that efferent pathways carry efference copy or corollary discharge. We have now shown, using three experimental techniques, that two second order neurones in the house cricket, *Acheta domestica*—the lateral and medial giant interneurons (LGI and MGI)⁶⁻⁹, the largest axons in the abdominal connectives—are under efferent control. We have also shown that the control blocks impulses in these neurones, and that the efferent inhibition is operative in the behavioural circumstances that would result in self-stimulation.

Recordings were obtained from free-moving animals by securing a pair of 50 μ m wires around one connective, anterior to the terminal abdominal ganglion¹⁰. Each animal was tethered by the recording leads, in a 12 $\times$ 12 inch arena with sand over the floor. The stimulus was a continuous 12 Hz sine wave contaminated by distortion in the output of the speaker.

Good control of stimulus parameters and clear identification of the LGI and MGI were achieved in restrained but minimally dissected crickets. After removal of the wings and the leg at the coxotrochanteral joint, each cricket was mounted ventral side up, and a flap of abdominal cuticle was

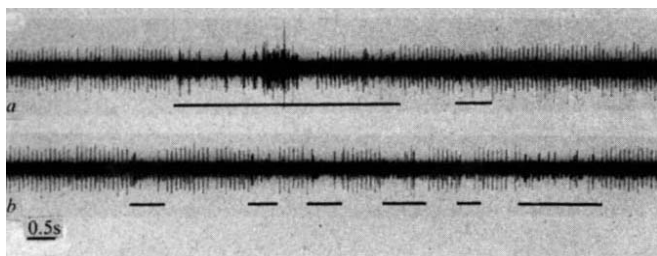


FIG. 1 Inhibition of giant fibres during running and grooming in a free-moving cricket with implanted electrodes (periods of running (a) and grooming (b) behaviour are marked with black bars). The animal was stimulated with 12 Hz tone which elicited giant fibre activity at each cycle; this activity was suppressed during episodes of behaviour.

removed. Silver wire electrodes surrounded by petroleum jelly recorded electrical activity in the two connectives independently.

Intracellular recordings were obtained from the dendrites of the MGI, as described elsewhere¹¹. Briefly, the animal was dissected from the dorsal side and the terminal ganglion was supported by a spoon-shaped holder; suction electrodes were attached to one abdominal connective and to the ipsilateral main leg nerve of the metathorax. A micropipette filled with Procion yellow¹² or potassium acetate was inserted through the sheath, and the neuropile was probed for the large dendrites of the MGI, which were identified by their responses to sensory stimulation and their electrical characteristics^{6,7,11}.

In free-moving preparations large spikes occurred with every cycle of the low frequency sound stimulus. During each bout of running the large units were silenced, although the stimulus continued unchanged (Fig. 1a). The inhibition was also correlated with other movements of the metathoracic legs, such as grooming (Fig. 1b). The experimenter's taped verbal account of the animal's behaviour is not a precise measure of the timing and specificity of the effect, but spike activity was monitored through an audio channel throughout each experiment and the results were unequivocal.

In restrained, minimally dissected preparations the LGI and MGI are reliably identifiable in the extracellular record. A touch to the head or thorax elicited running, that is vigorous waving of the leg stumps. In a quiescent animal, a tone pulse of 600 Hz and moderate intensity elicited a burst of spikes. The response was inhibited profoundly during running episodes (Fig. 2). On average, the response to strong stimuli (90 dB) was diminished less than the response to weaker stimuli (80 dB), but within this range of intensities the units were sometimes silenced completely.

In the restrained and highly dissected preparations used for intracellular recording, the MGI received inhibitory post-synaptic potentials (i.p.s.p.s.) during spontaneous bursts of spikes recorded from the ipsilateral metathoracic main leg nerve. During spontaneous motor activity, recordings from the dendrites of the MGI showed a hyperpolarisation of as much as 8 mV, which was maintained throughout the motor activity and sometimes extended beyond it (Fig. 3b). The MGI was often inhibited before the first observable motor spike. It has been suggested that efferent control of the lateral giant interneurone of crayfish is mediated by presynaptic inhibition¹². Many of our results might have been interpreted in a similar way since there was often a considerable decrease in excitatory end plate potential (e.p.s.p.) amplitude without obvious i.p.s.p.s. In other cases, however, i.p.s.p.s. were clearly recorded in the MGI dendrite, thus excluding a purely presynaptic mechanism. We could not demonstrate any direct correlation between unitary i.p.s.p.s. and motor nerve spikes. We conclude that the linkage between motor neurone activity and MGI inhibition is indirect, possibly provided by

an inhibitory neurone which receives excitatory input in common with the motor neurones.

The source of the descending inhibition was localised by selective cutting of interganglionic connectives. Inhibition was blocked by bilateral connective section anywhere between the third thoracic and terminal abdominal ganglia, but not anterior to the metathoracic ganglion. Thus the metathoracic ganglion is sufficient to produce inhibition. The possibility of additional inhibitory influences descending from more rostral centres remains open.

Most inhibitory influence on the LGI and MGI was abolished by sectioning of the connective ipsilateral to the axon of interest, while cutting the contralateral connective had a small effect. In contrast, the smaller ascending giant interneurons which fire tonically receive their inhibitory control equally from the two connectives. This pattern of connections correlates well with the pattern of sensory connections of the same neurones and with their dendritic field shapes: the LGI and MGI receive virtually exclusively ipsilateral excitatory input from the cerci and have unilaterally distributed dendrites, while the smaller, tonically active units receive bilateral input and have bilaterally distributed dendrites⁷.

Another inhibitory effect, which was completely independent of the thoracic centres, was also observed. When stimulated to run by a puff of wind aimed at the body, a reduction in the response to a standard tone could be detected even after severing all connections between the terminal ganglion and the remainder of the nervous system. This reduction in response might be due to lingering air movements or inhibitory circuits within the terminal ganglion.

Although the exact function of the giant fibres in controlling evasive behaviour has not been determined⁹, powerful stimulation of cercal receptors, which excites the giant fibres

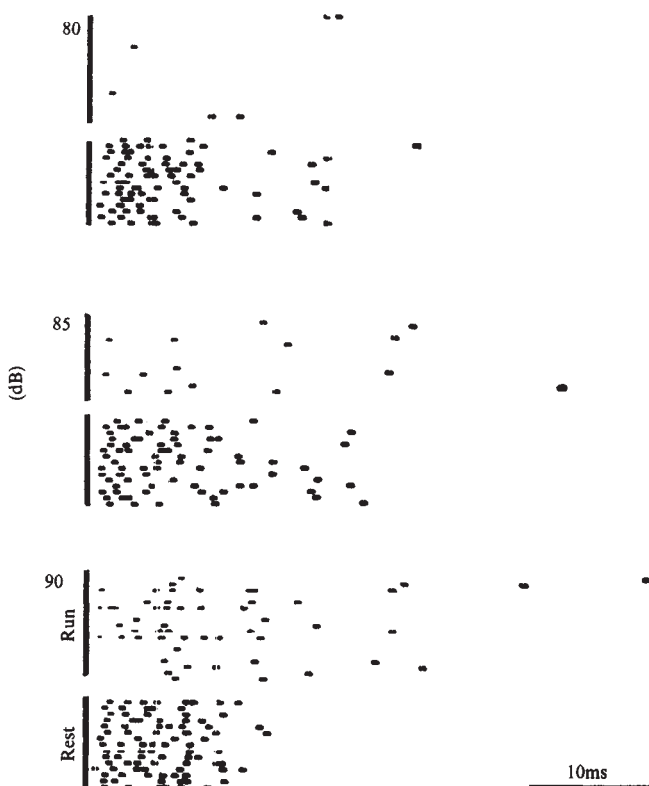


FIG. 2 Response of the two largest interneurons (LGI and MGI) to tones during periods of motor activity and rest. Each dot indicates the occurrence of a single spike, each row a single trial. For each stimulus intensity, indicated in the upper left hand corner, fifteen repetitions during touch-elicited running (above) and fifteen repetitions during rest (below) are shown.

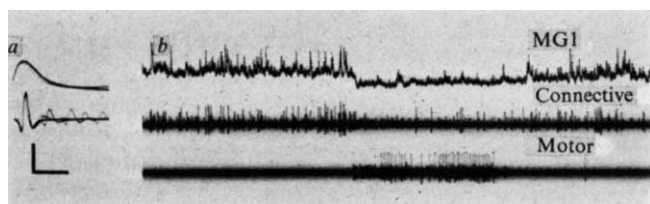


FIG. 3 Inhibition of the MGI during motor activity. (a) demonstrates that spikes recorded from the impaled neuron (top trace) are correlated 1:1 with spikes recorded extracellularly from the connective anterior to the terminal ganglion (lower trace). (b) shows that a spontaneous burst of motor activity (bottom trace) is correlated with a hyperpolarisation of the MGI (top trace) and a decrease in tonic activity in other giant fibres recorded extracellularly (middle trace). Calibrations: vertical a, upper, 40 mV; b, upper, 8 mV; horizontal a, 12 ms; b, 200 ms.

as a group, often elicits escape jumps. Therefore, excitation of the cercal receptors such as may occur during grooming or locomotion, might elicit evasive behaviour which would be inappropriate because the stimulus resulted from the animal's own behaviour and not from environmental events. On the other hand, escape is a high priority behaviour, and is not likely to be blocked completely during grooming or casual locomotion. In fact, the efferent inhibition can be overcome by strong stimulation (Fig. 2). Thus it seems that these neurones' thresholds are modulated to preserve the significance of their spike activity during behaviour. This could be interpreted as a simple example of the functioning of efference copy or corollary discharge.

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Chloride-related Depolarisation of Crayfish Muscle Membrane Induced by L-Glutamate

THE action of L-glutamate is localised at the junctional region of the muscle membrane in Crustacea and mimics that of the neurotransmitter in various characteristics. It is therefore possible that L-glutamate is the excitatory transmitter of the crustacean neuromuscular junction^{1,2}.

The excitatory junctional potential (e.j.p.) and glutamate potential induced by iontophoretic application of L-glutamate require Na but not Cl (refs 3, 4), but Cl was essential for depolarisation induced by the drug in the present experimental conditions.

The experiments were carried out on the opener muscle of an isolated walking leg of the crayfish (*Cambarus clarkii*). The experimental procedures, conditions and physiological solution were almost the same as those described elsewhere⁵. Propionate (Tokyo Kasei Kogyo, Co.) was used as the non-permeable and inert ion⁶⁻⁸ to replace all the Cl in the modified Van Harreveld's solution. To determine changes in resting potential, a coarse microelectrode filled with 3 M KCl was used as an indifferent electrode. Experiments were usually performed at room temperature (21°–22° C).

When L-glutamate (10^{-4} M) was added to normal medium, the muscle membrane was depolarised by about 10 mV after 1 min, and began to repolarise but stayed at a depolarised level a few minutes later, while the e.j.p.s. gradually disappeared at this time (Fig. 1Aa). On the other hand, when Cl was removed from the medium, the membrane was not depolarised but hyperpolarised by the drug (Fig. 1Ab). The large e.j.p. attained after the removal of Cl abruptly disappeared after the drug application, in contrast to the decreasing disappearance of the e.j.p. in normal medium (Fig. 1Aa).

This suggests that Cl is required for glutamate depolarisation in this case, provided that propionate replaced with Cl is inert to the postsynaptic membrane (Fig. 1Aa,b). The lack of glutamate response in Fig. 1b, however, may not be due to the accelerated desensitisation, because when glutamate was quickly added by iontophoretic application, the drug could

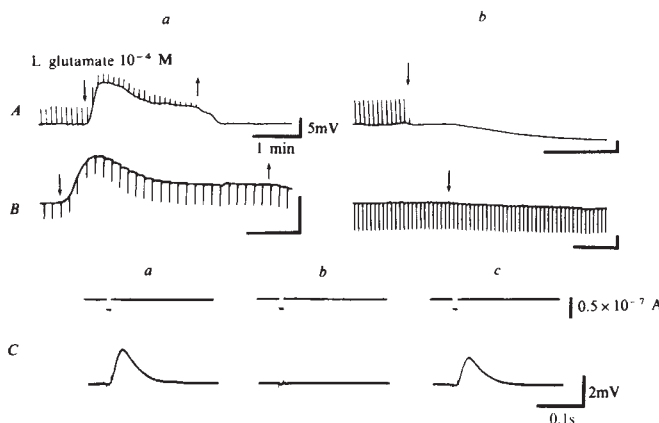


Fig. 1 Effect of chloride withdrawal on L-glutamate-induced depolarisation and input resistance. A: Excitatory axon was stimulated by a train of 8 impulses at 90 s^{-1} every 6 s, and the e.j.p.s. were displayed with a pen-writing recorder. The drug was added at the downward arrow by bath application and removed at the upward arrow. Calibration bars given for A and B are 1 min and 5 mV in all cases. a, Normal medium; b, 15 min after Cl removal. B: Two microelectrodes, one for recording and the other for passing current, were inserted into the middle of a muscle fibre. Electrotonic potential was produced by passing a current pulse of 2×10^{-8} A across the muscle membrane. C: L-Glutamate was applied iontophoretically at a junctional region after Takeuchi and Takeuchi². a, In normal solution; b, during withdrawal of Cl; c, after returning to the normal solution.

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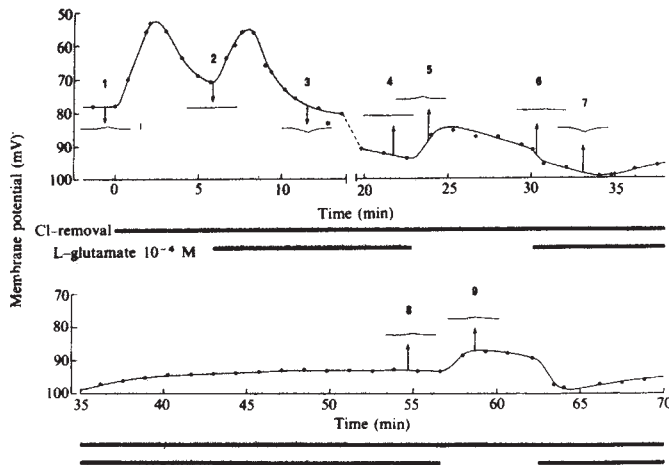


Fig. 2 Effects of L-glutamate on resting potential and inhibitory junctional potentials. The resting potential was monitored with a pen-writing recorder and the i.j.p.s that were induced by stimulating the inhibitory axon with a train of 8 impulses at 90 s^{-1} were displayed at each arrow by oscillographic means. Vertical bar at arrow 1 shows 1 mV for i.j.p., but 2 mV for a dotted i.j.p. The medium was perfused with a Cl-free solution at the upper bar and the drug was added at the lower bar by perfusing a preparation with a solution containing the drug. At arrows 7, 8 and 9, the same as arrows 3, 4 and 5 was repeatable, but 1 h after the Cl removal the hyperpolarising 'i.j.p.' was not observed further after drug application.

not produce any potential with Cl removal (Fig. 1C). This mode of drug action differed from that previously reported and that of the neurotransmitter²⁻⁴. There should therefore be another site of action for L-glutamate at which Cl is playing an essential role.

Effective resistance increased with depolarisation after the drug application (Fig. 1Ba). L-Glutamate, however, produced neither a depolarisation nor an increase in effective resistance after Cl removal (Fig. 1Bc). A simple interpretation of these facts is that Cl may carry all the depolarising current induced by L-glutamate without any considerable conductance increase, although this is rather complex because of the underlying non-linear relationship between the current and the voltage^{9,10}. Since the drug depolarised the membrane well beyond the Cl potential (Fig. 1Aa) that is usually found close to the resting potential¹¹⁻¹³, this amount of depolarisation cannot be brought about by an increase of Cl conductance alone, if at all. The Cl therefore must be 'pumped out' against an electrochemical gradient in this case. The experiments shown in Figs 2 and 3 may support this theory, although a possibility that Cl is indispensable for the depolarisation induced by other ions is not completely excluded.

With Cl removal the membrane potential was largely depolarised but began to repolarise after a few minutes (Fig. 2), while a depolarising i.j.p. induced by repetitive stimulation of the inhibitory axon (arrow 1) disappeared several minutes later (arrow 2). In this condition, L-glutamate still produced the depolarisation, presumably because some amount of internal Cl remained. About 2 min after drug application, the membrane began to repolarise and became hyperpolarised beyond the original level. During repolarisation, a large hyperpolarising 'i.j.p.' appeared in spite of a Cl-deficient condition as shown at arrow 3. The 'i.j.p.' became smaller in size with the hyperpolarisation of the membrane and disappeared at arrow 4. Since the i.j.p. is induced by selective conductance increase of Cl (refs 11, 14), this experimental result indicates that a concentration gradient of the ion across the membrane has become high at least at the synaptic region after the drug application. The greater amount of external Cl beyond that predicted from the Nernst equation should be pumped out and retained there for a while. A considerable amount of Cl can be expected to remain inside the muscle fibre even in Cl-free medium¹⁴⁻¹⁶.

When L-glutamate was removed from the medium, the i.j.p. began to go positive (arrow 5) and then diminished at arrow 6, suggesting that the amount of external Cl 'pumped out' decreased after withdrawal of the drug. Although the possibility that K or propionate may become permeable when the inhibitory synapses work under the glutamate action has not been excluded, this experimental fact can more reasonably be accounted for by the stimulating action of the drug for the 'Cl-pump' at the junctional region than by the interaction between the drugs.

When the temperature of a preparation was varied by perfusing it with cold or warm solution, glutamate potential altered greatly depending on the temperature (Fig. 3). With cooling, the potential became smaller and finally disappeared when cooled below 15°C in this preparation. The potential reappeared and was restored to original size by warming the muscle fibre. The peak time of these potentials showed little change in the course of this procedure^{6,17,18}. Essentially the same result was obtained in a case where the drug was applied by the perfusing procedure. The temperature at which the glutamate potential disappeared depended on the preparation and the quantity of the applied drug, ranging from 15° – 19°C .

From these experimental results it seems likely that the Cl-related depolarisation produced by L-glutamate was brought about by an 'electrogenic Cl pump', at least in part, which might be loosely coupled with the 'Na pump'. The slow hyperpolarisation after the drug application and the depolarisation after removing it in Fig. 2 suggest a hyperpolarising action of the drug in addition to the depolarising action. The depolarising action would be dominant in a normal medium. In this connection, it is interesting that this type of active transport has been found in various tissues of many kinds of animals¹⁹⁻²⁴. It is also likely that this type of activity may prove to be of importance in the control of the synaptic activities or the long-term reaction in the muscle^{25,26}.

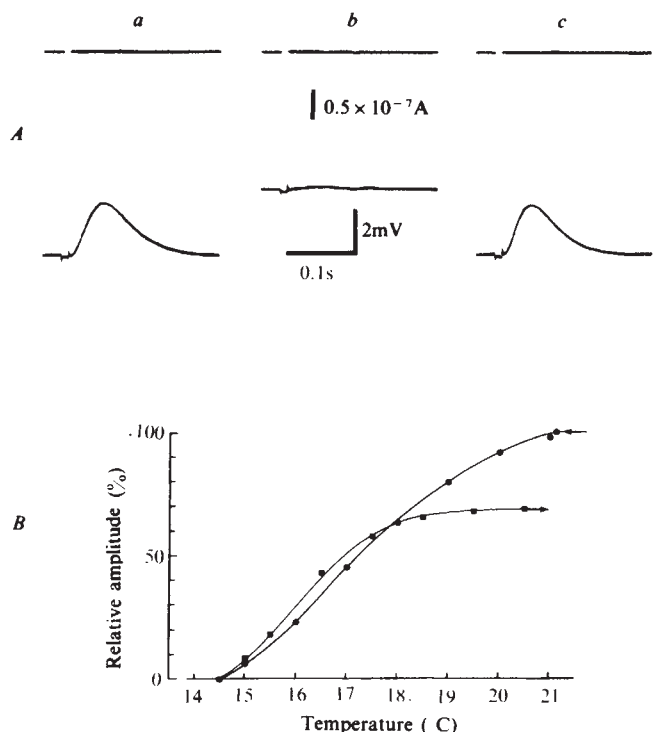


Fig. 3 Effect of temperature on glutamate potential. L-Glutamate was applied iontophoretically at a junctional region every 12 s. The preparation was perfused with cold or warm solution and its temperature was read on a thermometer inserted close to the preparation in the bath. A: a, at 21°C ; b, below 15°C ; c, after rewarming the preparation to 21°C . B: Relative amplitude of the glutamate potentials plotted against temperature. Arrows show experimental procedure.

Some dissociated characteristics between the e.j.p. and the glutamate potential should be subjected to further investigation so that L-glutamate may obtain a definite qualification as a candidate for the excitatory transmitter at the crustacean neuromuscular junction.

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microelectrodes and recordings were made of potential differences between electrode pairs. Changes in potential were observed when there was a change of electrode position relative to the retina, or when the retina was stimulated. Stimulation consisted of constant current pulses applied across the edge followed by light flashes perpendicularly incident to the receptor axes.

The records of Fig. 1 were obtained from two electrodes on a line at right angles to the edge. One electrode remained fixed, as the edge was advanced upon it; the other was always adjusted to a given reference position at the receptor tips. The bathing medium contained aspartate ions to simplify the potentials⁷. Little response to light was seen when the retinal electrode was 30 μm deep (Fig. 1a). At 45 μm it registered a small response which long outlasted the stimulus (Fig. 1b). This region of the retina contains mostly rod outer limbs. At 60 μm and beyond (Fig. 1c-g) the electrode was among both rods and cones and recorded two components, the prolonged one previously seen and a fast one with a time course matching the stimulus. Individually the two components resemble intracellularly recorded rod and cone receptor potentials⁸.

The relationship between dark voltage and photovoltage was examined in the experiments of Fig. 2. The electrodes remained

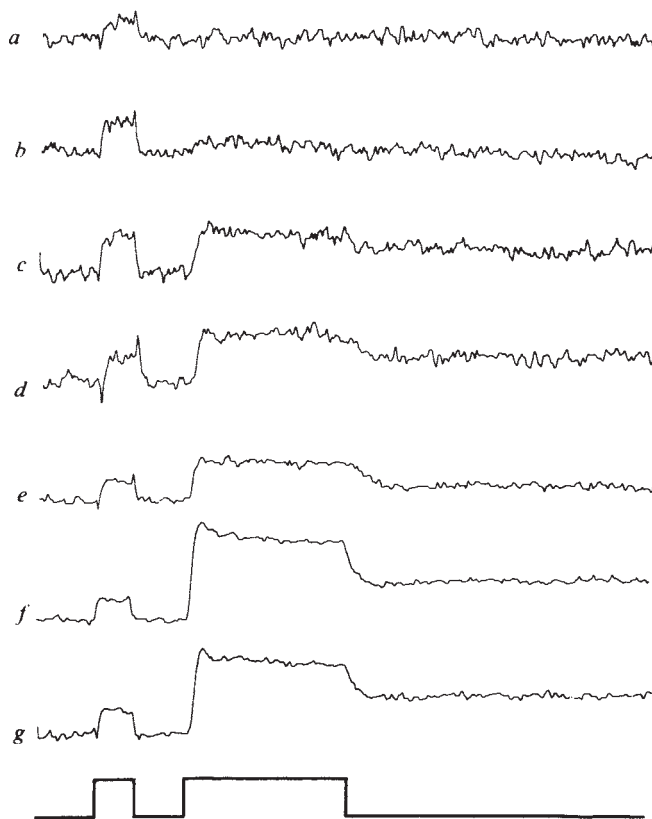


Fig. 1 Average voltages across receptor layer due to current pulses and responses to light flashes, repeated two to four times at each depth. a, 0–30 μm ; b, 0–45 μm ; c, 0–60 μm ; d, 0–75 μm ; e, 0–90 μm ; f, 0–105 μm ; g, 0–120 μm . 0 μm corresponds to the receptor tips. Calibration deflections mark the occurrence of current pulses (duration: 240 ms), and light flashes (1 s). The amplitude of the deflections corresponds to 15.6 μV (a–c), 31.3 μV (d), and 62.5 μV (e–g). Current pulses were 5.5 μA (a–e) and 1.7 μA (f–g). Upward deflection recorded when current flowed receptor to ganglion side. Light flashes: 561 nm, 3.7×10^{13} $\text{h}\nu \text{ cm}^{-2} \text{ s}^{-1}$, incident perpendicularly to the receptor axes. This light intensity produced a nearly saturated cone response but had no observable effect on the rod response when repeated at intervals of more than 40 s. Bandpass: 0–16 Hz. Electrode tips: about 2 μm , 8 M Ω , filled with Ringer. Ringer: NaCl 72, KCl 1.8, CaCl_2 0.1, MgSO_4 0.1, NaH_2PO_4 2.5, Na_2HPO_4 10, glucose 10, monosodium aspartate 10 mmol l^{-1} (pH = 7.2–7.3).

Extracellular Currents from Frog Photoreceptors

EVIDENCE has been presented to show that dark current flows around vertebrate photoreceptors^{1,2}. The outer limb is a sink for this current; the remainder of the receptor is a source. The effect of light is to reduce the current by increasing the resistance of the outer limb membrane^{3,4}. Some authors have, however, suggested complications to this hypothesis^{5,6}. The problem was therefore reinvestigated for the frog, *Rana esculenta*.

Our methods were modelled on those described by Hagins *et al.*^{1,2}, but instead of retinal slices, whole retinas were mounted on a holder consisting of two perspex plates hinged by a collodion film. When this holder was folded, the receptor edge of the retina protruded through a 1 mm long slit in the collodion. The holder was placed between two pools of Ringer on a microscope slide, and the receptor edge could be viewed by infrared microscopy. The edge was moved onto an array of

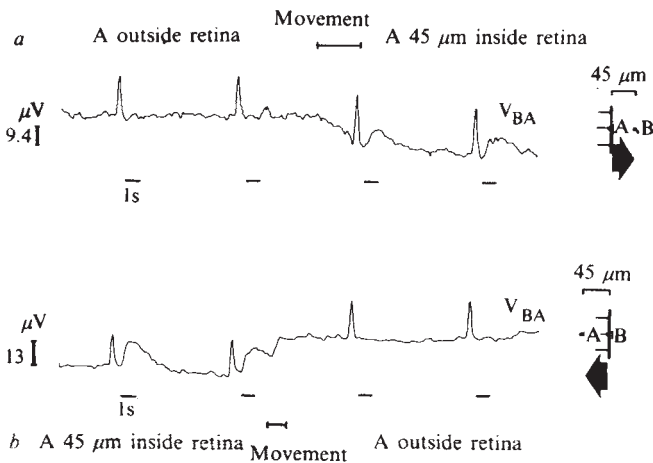


Fig. 2 Continuous records showing responses to light flashes and DC changes accompanying changes in the position of the electrodes relative to the retina. Position of electrode tips, B and A, before movement and direction of movement of the retinal edge is indicated to the right of each record. Current pulses flowing receptor to ganglion side precede the light flashes and produce voltage 'spikes'. Downward deflection corresponds to positivity of A. Light flashes: 1 s, 561 nm, 3.7×10^{10} $h\nu$ cm^{-2} s^{-1} . Horizontal lines mark the duration of the flashes and of the movements. The light intensity saturated the rod response without eliciting any noticeable cone activity. Bandpass: 0–1.6 Hz. This severe filtering lengthens the rise time of the rod response but has been shown not to affect its maximum amplitude. Electrode tips: 1–3 μm , 6–16 m Ω , filled with Ringer. *a*, A changes position, 0→45 μm from receptor tips. Ringer: see Fig. 1 except 82 mmol l^{-1} NaCl and no aspartate. *b*, A changes position 45→0 μm . Ringer: see Fig. 1 except 10 mmol l^{-1} monosodium glutamate instead of aspartate.

stationary throughout and the edge was moved so that one electrode (A) entered the retina (Fig. 2*a*) or came out of it (Fig. 2*b*) while the other (B) was always outside. For currents originating in the retina, it was as if B always remained at the receptor tips, since within experimental error the region outside the retina was isopotential with the edge. The results shown are typical of more than seventy experiments, some performed with standard Ringer (such as Fig. 2*a*), some with aspartate or glutamate Ringer (such as Fig. 2*b*). They represent the potential recorded by B minus the potential recorded by A (V_{BA}). Entry of the electrode into the outermost 45 μm of the retina always produced a downward shift in the DC level (A goes positive relative to B) followed by the appearance of upward-going photoresponses, the amplitudes of which were never greater than the shift. When the electrode came out, there was an upward DC shift and the responses disappeared.

Two types of effect were seen when movements of the edge brought the retinal electrode to the region of the outer limiting membrane (about 90 μm). In about one third of the cases the retinal electrode recorded increasing positivity (usually between 50 and 100 μV), as would be expected if a dark current flowed from the inner regions of the receptors to their outer limbs. But in most cases, during the last stages of the movement the increase in positivity was interrupted by a negative shift of the DC level (a millivolt or more), but most of the shift proved transient. When the retina was moved off the electrodes, no big shifts were seen. Then about two thirds of the records showed a decrease in positivity of A matching the diminution of the photoresponses. Probably the big DC shifts arise when an electrode tip disrupts the tightly packed structure of the inner receptor layer⁹.

In a few experiments giant, unstable photoresponses, up to a millivolt, were recorded from electrodes reaching the region of the outer limiting membrane. But the voltages produced by current pulses flowing parallel to the receptor axes (in the so-called 'radial' direction) remained the size expected for the

electrode position, suggesting that the major part of the large photoresponses was not generated across the radial extracellular resistance. Similar giant responses have previously been obtained at various depths in the frog receptor layer¹⁰. They probably arise in much the same way as the large extracellular responses that can be recorded when an electrode contacts a single spinal motoneurone¹¹. In any case giant responses, like the transient DC shifts, mask the relationship between radial dark voltages and photovoltages, and both types of effect have been excluded from subsequent analysis.

Measurements of the potential difference between two points along the extracellular path parallel to the receptors, as in the experiments of Fig. 2, do not allow components from local sources and sinks to be distinguished from those of remoter origin. But if potential measurements are made at three successive points (C, B and A) in a radial direction, then given certain assumptions, the potential differences, V_{BA} and V_{CB} , can be used to compute I , the current arising or disappearing between C and A^{2,12}.

$$I = (i/v_{BA}) \{V_{BA} - (v_{BA}/v_{CB}) \cdot V_{CB}\} \quad (1)$$

where v_{BA} and v_{CB} are the voltages generated by constant current pulses of magnitude i applied to flow along CA.

Equation (1) was used to determine photocurrents and dark currents from the photovoltages shown in Fig. 1 and from the corresponding dark voltage changes (not shown) produced by changes in electrode position. The results can be seen in Fig. 3.

Consider first the leading edge of the photocurrent. About 680 current units were generated in the layer 30–60 μm deep, while about 370 disappeared in the layer 60–90 μm , and 420 in the layer 90–120 μm . If about 110 units were produced in the layer 0–30 μm but could not be detected owing to the

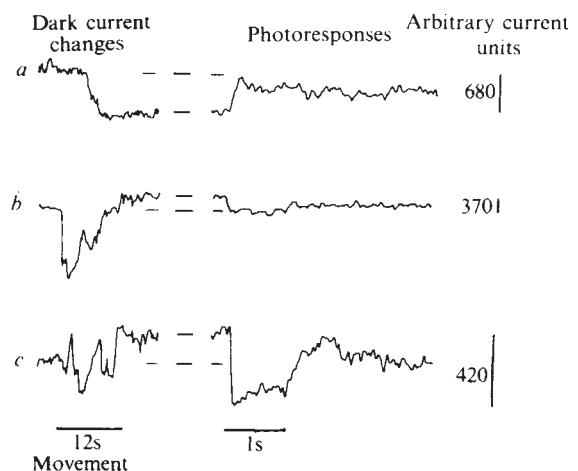


Fig. 3 Current sources and sinks 30–120 μm from receptor tips. Experimental details: see legend to Fig. 1. The dark voltage changes for each 15 μm change of electrode position were measured directly. The voltages across each 15 μm layer due to current pulses and photoresponses were obtained by subtracting the successive records of Fig. 1 from each other. The potential differences across two adjacent 15 μm layers were scaled to compensate for resistance differences between the layers, subtracted from each other and converted to currents according to equation (1). Currents at three depths in the receptor layer are shown: *a*, outer limb region, 30–60 μm ; *b*, inner limb and myoid, 60–90 μm ; *c*, nucleus and synaptic region, 90–120 μm . For a given depth the dark current changes are compared with the photoresponses plotted on the same scale. The scale is indicated in arbitrary current units to the right of the photoresponses. Arbitrary current units are used because of problems in accurately defining the geometry of the retinal edge. The area of the exposed edge was of the order of 1 mm^2 . The dotted lines indicate the dark current before and after the retinal movement with its transient disturbances. Upward shifts represent current sources, downward shifts sinks. Bandpass for dark current changes: 0–1.6 Hz; for photoresponses, 0–4 Hz.

low resistance of the layer, all the photocurrent is accounted for and this must have arisen from the receptors, since up to about 90 μm no other cells are present. Likewise the dark current up to 90 μm can only have originated in the receptors. Its magnitude is in agreement with that of the photocurrent but is opposite in sign. Such a result provides evidence for the view that receptor photocurrents represent reductions of dark current, at least in the aspartate-treated retina.

For the layer 90–120 μm , there is a discrepancy between the size of the dark current actually observed and that needed from the layer to balance the sinks in the outer limbs. This implies that the layer contains not only receptor sources of dark current but also non-receptor sinks balanced by sources still deeper in the retina. Non-receptor dark currents account for a further discrepancy in Fig. 3c. Thus the net dark current source (receptor source + non-receptor sink) is smaller than the leading edge of the photocurrent (receptor alone). In other experiments, sinks of dark current in the inner receptor layer were even more pronounced, especially if no aspartate was added to the Ringer solution.

Figure 3 shows that, in addition to non-receptor dark currents in the innermost receptor layer, there are non-receptor photocurrents. The response waveforms of Fig. 3a and b match each other but are different from that of Fig. 3c. The result is consistent with the idea that there is a source of photocurrent in the layer of Fig. 3c, which develops slowly and reaches its peak after the cone and rod sinks have passed theirs. Possibly the source is in the outer ends of the Müller cells and gives rise to what has been called slow PIII in other vertebrates^{13,14}.

The existence of variable non-receptor sinks and sources of current in the receptor layer could perhaps explain some of the differences between the results of various studies. Further analyses of receptor function based on extracellular current measurements, especially those of the innermost receptor layer, will have to take non-receptor currents into account.

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Nematode growth factor

FREE-living nematodes, grown axenically in a chemically defined medium, might be of considerable interest as model systems for studying the fundamental aspects of genetics and differentiation of higher organisms.

Several species of free-living nematodes have been cultured serially in axenic media¹. Unfortunately, the chemically defined medium in current use, *Caenorhabditis briggsae* Maintenance Medium (CbMM)², does not support continuous growth unless supplemented with a 'growth factor' that can be derived from various biological sources. Conventional growth factor, thus prepared is proteinaceous and biologically highly active. When added as a supplement to the chemically defined medium it supports growth and maturation of the commonly used test organism *Caenorhabditis briggsae* within 3.5 (yeast ribosomes) to 5 d (heated liver extract) and continuous development for several generations, resulting in large final populations. The biological activity is associated with precipitate formation²⁻⁴.

It has been shown that *C. briggsae* has a nutritional requirement for sterols and haem^{5,6} that must be provided by the growth factor as they are not included in the basal medium. The proteinaceous portion of the growth factor has been substituted with some success by several commercially available proteins⁷. Substitution of the protein portion by heat stable material, such as glycogen, is also effective and as with growth factor, the biological activity is associated with the precipitated portion of the medium⁸.

Thus the current idea is that the growth factor consists of sterols, a haem moiety and organic material which might provide nutrient in the proper particulate form⁸.

I now wish to further clarify the nature of the growth factor for *C. briggsae*. My hypothesis is that growth factor contributes only two dietary essentials (sterols and haem) to the basal medium and that the third portion of the growth factor, whatever its chemical nature might be, serves only as a convenient vector of the haem component, which must be supplied in a particulate form. This implies that any material should be able to substitute for the third component of the growth factor, provided that it is not toxic to the nematodes, capable of 'carrying' an iron protoporphyrin complex, either by containing haem (such as a haem protein) or by binding an effective substitute (haemin chloride) and available to the nematodes in particulate form.

I have confirmed the growth promoting activity of ribosomes from yeast^{3,4} and *E. coli*³ and obtained the same activity with ribonucleoprotein (RNP) particles from bovine liver and HeLa cells. Pea seedling-RNP particles were unable to support growth but their activity was restored upon addition of haemin chloride. This supports the theory as most RNP particles washed only in low salt buffers, are easily contaminated with a haem protein (most probably a cytochrome⁹). *E. coli* ribosomes washed with 1 M salt are inactive, as could be expected.

In another set of experiments, several purified haem proteins were shown to be highly active when properly precipitated and added to the basal medium, containing sterols.

In a third series of experiments, iron binding proteins were allowed to interact with haemin chloride and then carefully precipitated and tested for their growth promoting activity. Some of these aggregates (especially ferritin-haemin and transferrin-haemin) proved to be as active as growth factors, derived from natural sources. Less specific iron-binding material as bovine serum albumin, albumen or conalbumin permits only limited development of *C. briggsae* with a longer generation time.

In the next set of experiments, the 'carrier' portion of the growth factor was substituted by the phospholipid lecithin. Egg lecithin was dispersed ultrasonically and haemin chloride

was allowed to interact with the phospholipid particles before addition to the basal medium containing sterols. It was expected that lecithin-haemin complexes might be formed (both are lipophilic), providing haemin in particulate form. Lecithin, supplied at a final concentration of 10 mg ml⁻¹ proved to be toxic. At concentrations of lecithin 1 mg ml⁻¹, haemin 50 µg ml⁻¹, *C. briggsae* developed continuously for several generations (Table 1).

Details of the above experiments will be given elsewhere. Although the results, reported here, do not allow definitive conclusions about the minimal chemical composition of the growth factor, they strongly support the hypothesis that the third component of growth factors, derived from natural sources, is primarily a matter of function (as a convenient

vector of haem) rather than of chemical structure. This suggests a trivial explanation for the failure of previous trials to elucidate the nature of the growth factor by means of sophisticated biochemical purification.

Conclusive proof that the iron protoporphyrin constituent in any particulate form is the active component in sterol supplemented medium, is given by the fact that a medium, composed of CbMM, sterols and precipitated haemin only, will support continuous growth and reproduction of *C. briggsae*. The experimental design was based on the apparent irreversibility of haemin precipitation in acid medium. When the haemin suspension is brought to pH 5–5.4, haemin does not redissolve appreciably. This pH range is still compatible with the acid pH tolerance of *C. briggsae*. The results of two experimental arrangements are represented in Table 2. In these experiments, growth, comparable with that supported by natural growth factors, has been obtained with concentrations of 25 to 100 µg haemin ml⁻¹. In general, there are only minor differences between the first and the second experiment, probably due to different pH and salt concentration (high concentration of salt and low pH are both unfavourable). Higher concentrations of haemin (200 µg ml⁻¹) inhibit fast growth in both experiments. Although the influence of salt cannot be excluded, indications from former experiments suggest that haemin itself becomes toxic at higher concentrations.

Up till now *C. briggsae* has been maintained axenically through 10 serial subcultures in this medium (haemin at 100 µg ml⁻¹; pH = 5.0) without change in growth rate. CbMM supplemented with sterols (50 µg ml), haemin chloride (50 µg ml⁻¹) and 3% v/v acetic acid (pH of final medium 3.5) supports also growth and maturation of the

TABLE 1 Biological activity of lecithin-haemin particles

Final concentration of supplement	Generation time (d)	Increase over inoculum after 28 d
Lecithin 10 mg ml ⁻¹	nm*	—
Haemin 10 µg ml ⁻¹		
Lecithin 10 mg ml ⁻¹	nm*	—
Haemin 50 µg ml ⁻¹		
Lecithin 1 mg ml ⁻¹	7–10	× 2†
Haemin 10 µg ml ⁻¹		
Lecithin 1 mg ml ⁻¹	6–9	× 250‡
Haemin 50 µg ml ⁻¹		

Experiment carried out in four replicates. Sterols were included at a final concentration of 50 µg ml⁻¹ (ref. 5).

* nm indicates non-maturing.

† Only a few larvae emerged but failed to develop.

‡ Thriving cultures; F₂ larvae produced.

TABLE 2 Growth of *C. briggsae* on chemically defined medium containing sterols and supplemented with various concentrations of precipitated haemin

Haemin µg ml ⁻¹	F ₁ generation time (d)	F ₂ generation time (d)	Population at 7 d	Population at 14 d	Population at 21 d	Egg mass at 21 d
First precipitation method. Final pH of complete medium = 5.0*						
	(16)			(8)	(8)	(8)
200	7.5 ± 0.15	uncertain	nd	20 ± 4	376 ± 95	absent
100	6.2 ± 0.1	12 to 14	nd	75 ± 11	1,127 ± 103	large
50	6.0 ± 0.1	12 to 14	nd	146 ± 17	1,583 ± 232	large
25	6.0 ± 0.1	12 to 14	nd	126 ± 19	1,140 ± 94	small
12.5	6.1 ± 0.1	13 to 14	nd	96 ± 22	567 ± 50	very small
6.25	6.0 ± 0.1	uncertain	nd	32 ± 5	154 ± 25	absent
3.1	6.0 ± 0.12	no F ₂	nd	24 ± 6	59 ± 9	absent
1.6	6.2 to nm	no F ₂	nd	8 ± 3	29 ± 6	absent
Second precipitation method. Final pH of complete medium = 5.4†						
	(20)		(8)	(8)	(4)	(4)
200	6.9 ± 0.4	uncertain	4 ± 1	25 ± 5	172 ± 97	absent
100	5.6 ± 0.15	11 to 13	21 ± 6	185 ± 46	2,109 ± 145	very large
50	5.2 ± 0.15	10 to 11	26 ± 3	790 ± 62	1,401 ± 358	very large
25	5.0 ± 0.1	10 to 12	36 ± 6	598 ± 53	662 ± 75	small
12.5	5.3 ± 0.2	no F ₂	21 ± 3	50 ± 7	67 ± 13	absent
6.25	7.5 ± 0.6	no F ₂	8 ± 1	16 ± 5	28 ± 7	absent
3.1	7.5 to nm	haemin dissolves again : no further growth supported				
1.6	nm					

Mean values ± s.e. are given where possible. The number of replicates is given in brackets. Replicates were grouped in sets of four. Culture tubes containing 0.25 ml of medium were inoculated with three newly hatched larvae and incubated at 21–22°C. F₁ generation time is measured as the time from the incubation of these larvae to the production of larvae of the next generation. F₂ generation time is defined as the time required for two consecutive reproductive cycles. Media are evaluated further by the size of the population originating from the three initial larvae (counts of the cultures in *t.t.o.*). The size of the egg masses produced eventually after 21 d of growth provides an additional criterium. nd, Not determined; nm, not maturing.

* CbMM, containing sterols (50 µg ml⁻¹) was acidified with 1 N HCl to pH = 3.5 and subsequently supplemented with haemin chloride from a stock solution to obtain a final concentration of 400 µg ml⁻¹; in these conditions haemin precipitated immediately. This medium was then mixed with equal amounts of CbMM containing sterols (50 µg ml⁻¹) pH 7.67, thus giving a complete medium composed of CbMM, haemin at 200 µg ml⁻¹ and sterols at 50 µg ml⁻¹ with final pH of 5.0. This medium was then diluted serially with CbMM, pH 5.0, containing sterols (50 µg ml⁻¹) to obtain the concentrations of haemin indicated. This precipitation method implies the inclusion of high salt concentration in the medium.

† Haemin chloride was pipetted from stock solution into Hcl 0.01 N (pH = 2.0) to give a final concentration of 400 µg ml⁻¹. The suspension thus obtained, was then mixed with equal amounts of CbMM at twice the concentration, pH 5.9, containing sterols (100 µg ml⁻¹). The resulting complete medium is then composed of CbMM at 1 × concentration, haemin chloride at 200 µg ml⁻¹ and sterols at 50 µg ml⁻¹ with final pH = 5.4. Serial dilutions were made from this medium with CbMM (containing sterols at 50 µg ml⁻¹) pH 5.4.

free-living nematode *Turbatrix aceti*. This organism has now already been maintained under aseptic conditions throughout nine serial subcultures.

Four main conclusions can be drawn from these findings. First, the growth factor for *C. briggsae* has been elucidated. This growth factor, it should be emphasised, contributes only two dietary essential components to the basal medium: sterols and haem. Second, as the haem constituent can be substituted effectively by precipitated haemin chloride, a chemically fully defined medium for the axenic cultivation of *C. briggsae* is now available. Third, the fact that the haem component must be provided in particulate form to be efficient indicates strongly that it is taken up by phagocytosis. This hypothesis is now being checked. Fourth, it is quite conceivable and even probable that the elucidation of the growth factor for *C. briggsae*, may have significance far beyond the study of this single species, as suggested by the fact that supplements promoting growth of *C. briggsae* have also a stimulatory effect on growth of many other nematode species. In this respect, the successful axenic cultivation of *T. aceti* on a chemically fully defined medium may be highly significant.

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Depression of Morphine-seeking Behaviour by Dopamine Inhibition

MANY of the direct effects of morphine have been attributed to serotonergic mechanisms¹ or to the catecholamines^{2,3}. The role of brain monoamines in morphine's capacity as a behavioural reinforcer has only recently been explored^{4,5}, and it has been found that the catecholamine depletor α -methyl-*p*-tyrosine (AMT) blocks behavioural reinforcement by morphine. Our own work, started before these reports appeared, strongly suggests that dopamine is implicated in this blockade. To study morphine reward we adopted the technique of Beach⁶ which uses the acquisition and retention of a goal box preference and thereby minimises non-specific effects by experimental drugs on performance.

Sprague-Dawley male rats (180–300 g) were tested for pre-training preferences in a Y-maze with two contrasting boxes. Morphine dependence was induced by gradually increasing doses from 5 to 20 mg kg⁻¹ intraperitoneally twice daily, during 7 d. Training started with a saline injection in their home cages and an immediate forced run in the Y-maze to their preferred box where they were confined for 1 h. This was followed 20 min later by injection of 20 mg kg⁻¹ morphine and a forced-run to their non-preferred box and 1 h confinement therein. Training by forced runs was achieved by blocking off the opposite alley. We assume the rats learned to associate the effects of the injection with the response of running to the respective box. Sixteen free-choice trials (all barriers open) were presented on training days 15 and 16 (acquisition). Three weeks later, during which no treatment was administered (except see below), the rats were again tested with sixteen free choice trials (that is, 'relapse')⁶.

Our results show that the rats acquired and maintained a preference for their formerly non-preferred, morphine-rewarded box (group C-Mor, Fig. 1), in full agreement with Beach⁶. Choices of the latter were significantly greater than those of a group receiving saline alone (group C-Sal; $P < 0.01$), or compared with their own pretraining preference scores. No difference was found between these morphine-trained rats and parallel control groups receiving the carriers for the various modifying agents used below (Kruskal-Wallis analysis of variance⁷; d.f.=6; $P > 0.90$). These groups were therefore combined and are represented in Fig. 1 as C-Mor. All other P values were derived by the Mann-Whitney U test, two tailed⁸. That the learned preference was not an expression of avoidance of the 'withdrawal' box was shown by another control group run only to the preferred box. These rats selected the latter in 52 and 69% of their trials during the acquisition and relapse tests respectively, thus exhibiting no avoidance of this box. We, therefore, assume that the learned preferences are mediated by the euphoric and (or) the abstinence relieving components of morphine reinforcement^{6,9} rather than by avoidance of the opposite box, in line with the findings by Kumar¹⁰.

AMT, which has been repeatedly shown to deplete both noradrenaline and dopamine^{11–13}, was administered to another group of rats (80 mg kg⁻¹ intraperitoneally every 12 h) during morphine training. This treatment significantly depressed acquisition of a morphine-box preference ($P < 0.05$) and completely blocked relapse (Fig. 1). Initial choices of the morphine box were still greater than the C-Sal control group, indicating that the AMT only partially blocked morphine reinforcement. Catecholamine depletion by 6-hydroxydopamine ($2 \times 250 \mu\text{g}$ intraventricularly, 2–4 d before training^{14–16}) in another group of twenty-one rats also significantly reduced acquisition and relapse similar to the AMT. Preferential depletion of serotonin by *p*-chlorophenylalanine¹⁷ (300 mg kg⁻¹ d⁻¹ for 3 d starting 5 d before morphine training and continued with 150 mg kg⁻¹ every other day during training) had no significant effect on preference scores in either test condition (Fig. 1).

Since AMT depletes both noradrenaline and dopamine, we tested to see whether one of these monoamines was more essential in mediating morphine reinforcement. Another group received the dopamine- β -hydroxylase inhibitor diethylthiocarbamate (DETC), which depletes noradrenaline while dopamine levels remain unaffected or even increase¹⁸. DETC (400 mg kg⁻¹ injected daily 1.5 h before the morphine-reinforced trial) had no effect on morphine-box preferences (Fig. 1).

These data suggested that noradrenaline depletion was not critical in inhibiting morphine reinforcement. The role of dopamine was assessed in further groups treated with haloperidol which presumably blocks dopamine receptors¹⁹. Doses of 0.25, 0.5 and 1 mg kg⁻¹ haloperidol were administered 20 min before the daily morphine trials in separate groups. The 1 mg injections completely blocked morphine

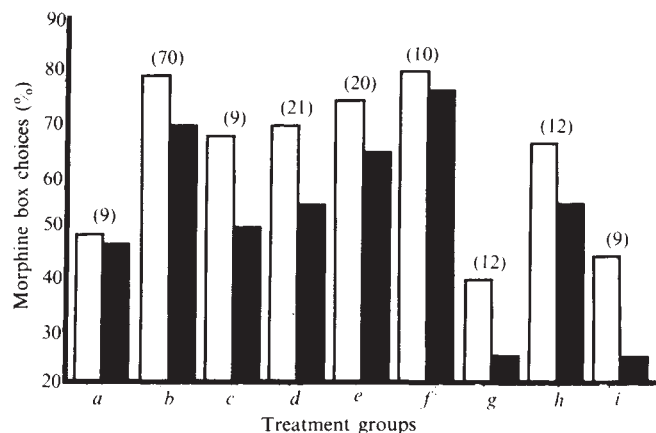


Fig. 1 Proportion of morphine-box choices (or non-preferred box as defined by pre-training preference trials) during free-choice tests after indicated treatments. Number of rats in each group shown in parentheses. Open columns, acquisition; black columns, relapse. a, C-Sal; b, C-Mor; c, AMT; d, 6-hydroxy-dopamine; e, p-chlorophenylalanine; f, DETC; g, 1 mg kg⁻¹ haloperidol; h, 0.5 mg kg⁻¹ haloperidol; i, AMT plus 0.5 mg kg⁻¹ haloperidol.

reinforcement whereas 0.25 and 0.5 mg produced a significant reduction compared with group C-Mor ($P < 0.05$ in each case). Combining the treatment schedules for AMT and 0.5 mg haloperidol in an additional group, however, significantly reduced morphine-box choices below that of either treatment alone ($P < 0.02$ in each case) and did not differ from the 1 mg haloperidol group (Fig. 1).

The possibility that the AMT interfered with original learning or later memory of the morphine-seeking habit was examined in twelve rats trained with morphine while treated with AMT exactly as before, but were re-addicted to morphine 1 week before relapse testing. These animals showed a mild decrease in initial choice tests, but during relapse testing they selected the morphine-rewarded box 70% of the time, indicating that AMT did not impair learning or memory as compared with the original AMT group. Neither did administration of the modifying drugs alone have any effect on preference scores, as shown by two groups receiving AMT or haloperidol but no morphine ($N = 12$ and 11 respectively). The selection of the non-preferred box by the AMT group was 42% and 35% for the haloperidol group, and did not significantly differ from the scores of group C-Sal.

The data indicate that interference with dopamine activity, in contrast to noradrenaline and serotonin, effectively reduces the behaviourally reinforcing effects of morphine. Dopamine reward mechanisms recently implicated in intracranial self-stimulation²⁰ may be involved; however, since AMT reduces certain abstinence symptoms in the rat²¹ and also makes morphine aversive²², the possibility is raised that the reward value of morphine was diminished by these effects as well.

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Appearance of Unusual Mitochondria in Rice Coleoptiles at Conditions of Secondary Anoxia

In rice coleoptile cells mitochondria can be formed and preserved without any notable signs of destruction in the absence of molecular oxygen¹⁻³. In this, rice coleoptiles are strikingly different from other plant tissues, whose fine cell organisation is degraded during even a short-term anaerobiosis⁴⁻⁶. In the experiments mentioned, however¹⁻³, the rice seeds were grown from the very beginning under oxygen-free conditions. This excluded not only the possibility of functioning of mitochondria but, probably also, their normal formation. One could therefore suppose that such mitochondria as yeast promitochondria^{7,8} do not contain all the carriers of the respiratory chain and possess an enhanced resistance to anaerobiosis. High resistance of cell ultrastructure to anoxia could be, on the other hand, secured by intensive energy output through glycolysis.

With this hypothesis in mind, we were interested to find out whether the cells of coleoptiles would lack resistance to anoxia after their mitochondrial apparatus and the whole system of aerobic metabolism had already begun to function under conditions of normal oxygen supply. For this purpose rice (*Oryza sativa*) seeds were cultivated from the beginning with a normal air supply; only after 6 d of growth were the plants placed in anaerobic conditions for 24, 72 and 120 h.

After corresponding exposure to an atmosphere of nitrogen (99.997% purity) sections of the lower part of coleoptiles, of leaves and of roots (1-2 mm from the tip) were fixed by the method of Karnovsky⁹, embedded in Epon 812 and studied under a Tesla BS-513 electron microscope.

Destructive changes in mitochondria and other organelles in roots take place 1-3 d after they have been transferred to anaerobic conditions (Fig. 1a, b). This did not occur with coleoptiles. Their mitochondria and other organelles remained intact even after 120 h exposure to the nitrogen atmosphere. Some of the mitochondria remained intact, without any substantial changes in form and size, producing only more regularly ordered cristae. Other mitochondria

were transformed to large organelles with multilayer cristae. These organelles often had an irregular form (Fig. 1d). They

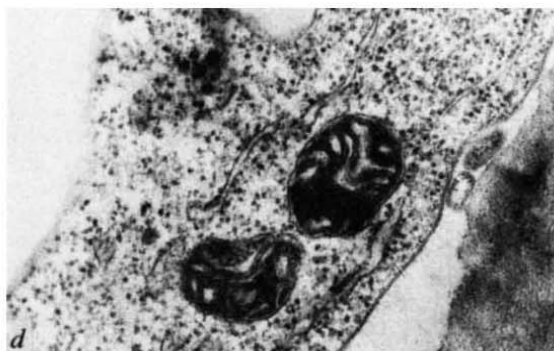
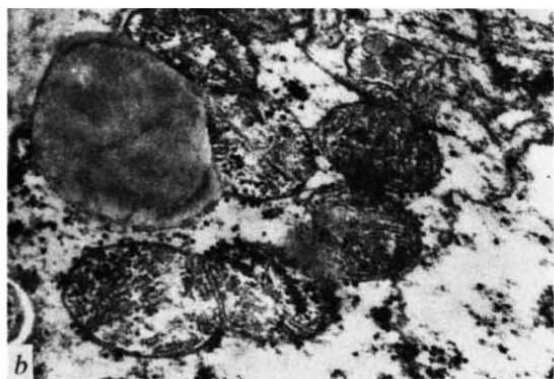
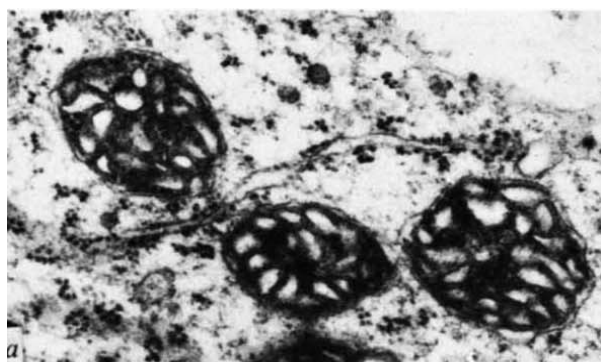


Fig. 1 Ultrastructure of mitochondria in cells of rice roots and coleoptiles in aerobic and anaerobic conditions. *a*, Root, 9 d after growth in aerobic conditions; *b*, root, 6 d of aerobic growth, then 3 d of growth in anaerobic conditions; *c*, coleoptile, 9 d of aerobic growth; *d*, coleoptile, 6 d of aerobic growth, then 5 d of growth in anaerobic conditions.

were 1.2–1.5 μm long whereas normal mitochondria are 0.5–0.6 μm long. The whole interior chamber of these mitochondria was filled with densely packed parallel cristae lying transversely to the long axis of the organelle.

Such mitochondria have not, as far as we know, been described yet in plant cells, for which layers of parallel cristae are not characteristic¹⁰. They are found in animal cells performing especially active work (cells of flight muscles in insects, myocardium of mammals)¹¹.

The appearance of these mitochondria, extremely unusual for plants, can hardly be attributed to the beginning of organelle degradation; it rather points to hypertrophy of the mitochondrial apparatus, arising in rice coleoptiles cells because of shortage in oxygen supply.

It is interesting that the mitochondria of leaves, which in contrast to coleoptiles do not start growing in the absence of O_2 revealed unexpectedly high resistance to anoxia: 3–5 d exposure of plants to nitrogen did not lead to their destruction.

High resistance of rice coleoptile cells to anaerobiosis is thus reflected not only in their ability to preserve mitochondrial apparatus when growing in strictly anaerobic conditions but also in especially active development of a number of mitochondrial cristae, possibly promoting more complete extraction of traces of oxygen from an environment which almost completely lacks it.

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Dynamics of a brachiating siamang [*Hylobates (Symphalangus) syndactylus*]

THE slow brachiation which is characteristic of the siamang [*Hylobates (Symphalangus) syndactylus*] and to a lesser extent the smaller gibbons [*Hylobates (Hylobates) spp.*] is often described as pendulum-like^{1,2}. This comparison is apt because both the pendulum and the gibbons rotate about a fixed overhead fulcrum and make use of gravitational acceleration to effect movement. Beyond these basic observations, there have been few attempts to elucidate the mechanical aspects of this unique form of locomotion^{2,3}. Analysis of 16 mm cine films of free-ranging and captive siamangs and gibbons reveals details of their locomotor behaviour that can be interpreted as precise adaptations for maximising the forward momentum gained from pendular movement.

This study is based on excerpts from several thousand feet of 16 mm film of wild siamang in Pahang, Malaysia and 100

feet of 16 mm film of captive siamang and gibbons on semi-natural islands in the Zoo Negara, Malaysia. Film was shot by the author with a Beaulieu R16B camera at speeds between 24 and 64 f.p.s.

The slow brachiation of the siamang involves a repeated series of movements. A representative cycle (Fig. 1) can be analysed in terms of two distinct phases involving the coordinated movements of trunk and legs as well as arms. The downswing phase begins as the animal releases the trailing hand. As the body swings forward and down with the legs extended, the free forelimb remains extended and sweeps through an arc behind the body. The upswing begins as the siamang flexes both legs and the free forelimb; midway through the upswing the forelimb is extended for the next grasp. During a complete swing, the trunk rotates through approximately 180° about a vertical axis.

In this form of locomotion, there are three ways in which the siamang can maximise its forward momentum: by maximising the change in kinetic energy on the downswing; by minimising the loss of kinetic energy on the upswing, and by minimising any lateral components in its momentum. Analysis of the siamang's posture in different phases of a single swing shows how the postural changes are adapted to maximise forward momentum in all three ways.

The total kinetic energy, and hence the maximum velocity acquired during the downswing, is proportional to the distance between the animal's centre of mass and the centre of rotation (in this case its hand). In a frictionless system, the increase in kinetic energy is proportional to the change in height of the centre of mass during the downswing ($\frac{1}{2}mv^2 = \frac{1}{2}lw^2 = mg\Delta h$). For a given arc (α) of

rotation, the vertical displacement of the centre of mass increases with the distance between the centre of mass and the centre of rotation [$\Delta h = \Delta r(\sin \alpha)$]. The siamang increases this distance on the downswing by extending its legs and its free arm (Fig. 1).

On the upswing, the siamang minimises the loss of momentum due to gravitational deceleration by approximating its centre of mass to the centre of rotation. This is accomplished by flexion of the legs and the free arm (Fig. 1). By decreasing the moment of inertia, this redistribution of mass should increase the rotational velocity of the siamang. This increase in velocity can be seen in stop-frame analysis.

For all of the kinetic energy acquired on the downswing to be converted into forward momentum, the centre of mass should travel in the same vertical plane as the centre of rotation. The siamang is able to limit lateral motion of the centre of mass between handholds by extensive rotation at the wrist, elbow, and shoulder (Fig. 1b).

In the motion of a simple pendulum all of the kinetic energy acquired on the downswing is subsequently changed to potential energy on the upswing so the pendulum retraces its path indefinitely. In a single swing of the siamang, the kinetic energy lost on the upswing is less than that acquired on the downswing because of the input of potential energy resulting from the redistribution of mass on the upswing. By the mechanisms described above, the siamang could theoretically acquire a net momentum from each swing.

In most forms of locomotion, an animal must exert a propulsive force against the environment in order to acquire a forward momentum. Although the brachiation of the siamang as described above probably involves some energy expenditure in supporting the body, only with the rotation of the trunk about the hand is there a suggestion of any work being performed against the superstrate. In instances when siamangs do not utilise this pumping mechanism, active rotation and brachial flexion appear to become more important. Further studies involving electromyography could possibly determine the nature of the muscular activity involved.

The analysis above illustrates a way in which a slowly brachiating siamang could maximise his net forward momentum from gravitational acceleration without exerting any propulsive force against the environment. In the same way that a child on a swing can increase the kinetic energy of that system by 'pumping', the siamang is able to maximise the kinetic energy of a single cycle by appropriately timed redistribution of its mass.

This work was supported in part by grants from the Hooton Fund, William F. Milton Fund and a biological training grant from the National Science Foundation to Harvard University, and the National Science Foundation to F. A. Jenkins, jun. All work in Malaysia was done in conjunction with the Institute for Medical Research, Kuala Lumpur and the Malaysia Game Department. I thank David Chivers for introducing me to the study of Malaysian primates. I thank R. T. Bakker, D. Fisher, R. Kay, J. Peterson and especially F. A. Jenkins, jun., for suggestions, comments and general encouragement and Laszlo Meszoly who prepared the illustrations.

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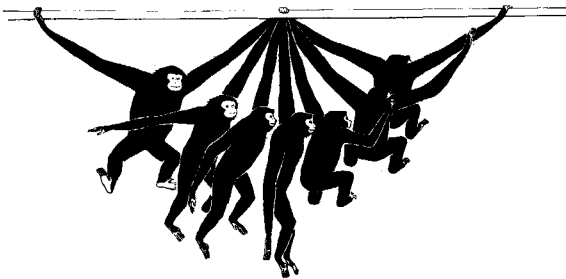
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a



b

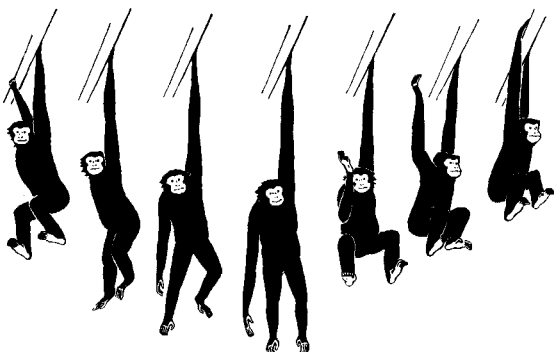


FIG. 1 a, Lateral view of a brachiating siamang, b, anterior view of a brachiating siamang. Figures rendered from tracings of 16 mm film stopped at intervals of $\frac{1}{24}$ s.

book reviews

Metamorphic rocks

Metamorphism and Metamorphic Belts. By Akiho Miyashiro. Pp. 492. (Allen and Unwin: London, September 1973.) £8.60.

THIS book is a welcome addition to geological literature; it gives a clear and positive approach towards an understanding of metamorphism, metamorphic belts and associated phenomena, and summarises much of the relevant literature.

The book is divided into three parts, of which the first presents basic concepts and methods of metamorphism and metamorphic petrology, together with a chapter where some major problems of metamorphic geology are objectively discussed. The second and third parts respectively concentrate upon two major aspects of metamorphic studies: the detailed examination of minerals and mineral assemblages, important to an understanding of petrogenesis; and the broader study of the geological relations of metamorphic terrains of various types, whence the nature and conditions of major tectonic processes and crustal evolution may be elucidated.

Part 2 summarises much descriptive literature on numerous sequences of progressive metamorphism throughout the world, and discusses their interpretation with regard to the compositional and reaction relations of minerals and mineral assemblages. Attention here is particularly focused on the abundant data available for regionally metamorphosed pelites (actually referred to as metapelites!), metabasites, and metaclasses; but there are also chapters on meta-limestones and contact metamorphism, together with a lucid account of the relations of eclogites and the eclogite facies.

In part 3, Miyashiro describes the general nature and distribution of regional metamorphic belts in North America, Europe, Japan and the Southwest Pacific. This is followed by chapters summarising the main features of metamorphic belts and examining their place in the tectonic, geochemical, and major petrological evolution of the Earth's crust. There is a particularly valuable summary and discussion of metamorphism and island arcs in the Japanese Islands. Many features are discussed in relation to the recent concepts of seafloor spreading and plate

tectonics, and these are shown to allow much interpretation of the origin of paired metamorphic belts. Much clearly remains to be learned about other metamorphic belts. Chapters on ocean-floor metamorphism and cataclastic metamorphism are included at the end of part 3; the former is a welcome addition to the usual ambit of metamorphism, but the latter is very perfunctory.

The division of the book into the three parts outlined above is a useful and logical one, but has led to some repetition and the presentation of some subjects (such as facies and facies series) in two sections. Chapter 3, in part 1, is almost a summary of the whole book. An appendix on the "History of the Study of Metamorphism", helpfully puts different approaches and viewpoints in perspective; Miyashiro does not do justice to his own major contributions.

The facies classification is used principally as a basis for the major subdivision of the P-T field of metamorphism, and Miyashiro adopts Eskola's eight facies together with the recently widely recognised zeolite and prehnite-pumpellyite facies. He laudably avoids erecting further facies which cannot be clearly and positively defined by paragenetic relations and which are unlikely to be adopted by the non-specialist—the use of petrogenetic grids is indicated for detailed facies subdivision. At the same time Miyashiro distinguishes, somewhat anomalously, some subfacies on the basis of facies series, even though paragenetic criteria may not be available. By placing the emphasis upon individual progressive grade sequences, broadly separated into three basic types but without detailed subfacies division, the approach adopted contrasts with that of the recent books by Turner (McGraw Hill, 1968) and Winkler (Springer-Verlag, 1967) where divisions into facies and/or subfacies form major aspects or objectives of presentation. This feature, together with the extensive treatment of the broader geological relations of metamorphic terrains, will probably make Miyashiro's book the most appropriate and readable one for many undergraduate teaching courses.

Miyashiro notes that he has not intended any advanced treatment of thermodynamic and structural aspects. Summaries are presented for the ther-

modynamic characteristics of metamorphic reactions, the significance of isograds, and the nature of petrogenetic grids and Schreinemakers bundles; but they are often too condensed to greatly help those who are not already aware of their import. Little attention is given to detailed petrography, or the relations of deformation and mineral growth. Miyashiro uses the old, rather vague, geological meaning of recrystallisation, instead of the more specific metallurgical one, recently employed by many metamorphic petrologists.

This book should prove useful not only to students and research workers studying metamorphism, but also to all those interested in the evolution of the Earth's crust.

B. HARTE

Desert plants drinking

A New Ecophysiological Approach to Forest-Water Relationships in Arid Climates. By I. Gindel. Pp. 142. (Junk: The Hague, 1973.) 40 guilders.

THE title of this book constitutes a claim to originality and that claim is amply justified within its pages. Gindel has taken a refreshingly novel look at the water relations of arid land plants. He has selected each of the anatomical and physiological properties which are normally associated with xerophytism, has systematically demolished entrenched assumptions and has constructed in their place a new and very personal philosophy of the xerophyte.

Some aspects of this reconstruction are more convincing than others. For example, he shows that many xerophytes have as many stomata on their upper leaf surfaces as they do on their lower surfaces; contrary to expectation, some xerophytes open their stomata during the night; following the exhaustion of available soil water, some xerophytes maintain turgour by the absorption of mist and dew, particularly during the night. Less convincing is Gindel's suggestion that water and even ions absorbed by stomata can be transferred to the soil through the roots, thus maintaining soil moisture levels beneath vegetation. He has not dealt adequately with the alternative explanation which relates the higher soil moisture beneath vegetation to the modified microclimatic conditions in such situations. Other, broader issues, such as overall transpiration rates in

xerophytes and even the function of transpiration in general are discussed, but no firm conclusions are reached.

The book is not easy to read. The language lacks fluent style, and, worse, many interesting thoughts are buried in a plethora of rather trite information. Often it is difficult to distinguish between the ideas for which Gindel has some experimental data and those which are purely speculative. The diagrams are embarrassingly crude and misspellings and misprints common.

In general, one is left with the impression that the book was prepared and produced in a hurry, which is sad because many of the ideas which are presented in it are stimulating and iconoclastic.

PETER D. MOORE

Life in space and time

Biogeography: An Ecological and Evolutionary Approach. By C. B. Cox, I. N. Healey and P. D. Moore. Pp. viii+179. (Blackwell Scientific: Oxford, 1973.) £3.00.

THIS is a very attractive book which is well illustrated and written in a lively and engaging style, so that it is likely to appeal to students at universities for whom it seems to be intended. But as with so many new textbooks, it tends to skim the cream and omit much of the milk from which the cream rises. It is fair to ask if such books are a proper diet on which to nurture a critical appreciation of an essentially descriptive and analytical subject, for this is surely what biogeography really is. It is the branch of science concerned with the study of distribution on a geographical scale of both fossil and living organisms and the communities and ecosystems of which they form a part. It cannot be conceived as including ecology and cytogenetics without destroying the meaning of words.

The authors have too broad a definition of their subject, at least when attempting to present it so summarily. With only 179 pages at their disposal, which includes numerous maps, drawings and photographs, the treatment is inevitably often superficial, even to the point of being misleading. A model of what is really needed for universities is provided by Stanley Cain's *Foundations of Plant Geography* (Harper, New York and London, 1944), an outstanding contribution to biogeography which is surprisingly not even mentioned in the references. In fact, the chapters on life on small islands and on continental drift approach this quality and present lucidly both the essential evidence and the arguments so that they serve to emphasise the weaknesses in other chapters. For example, the account of

soils is unbalanced. What is clearly a podzol, though this is not stated, is described on page 49 and gives an impression that most soils conform to this basic structure. The process of podzolisation is described subsequently. Surprisingly in a book of this title, there is no account of the great latitudinal belts of zonal soil types and no discussion either of the relationship between these and the major ecosystems of which they are a part, or of the conceptual parallelism between zonal soils and vegetational climax. Surely if a book must be so concise, it is these fundamental relationships and ideas which should be included and illustrated with a few carefully selected and adequately presented examples. Moreover, examples must be recognisable as such and not be mistaken for generalities. In fact, this technique is used in the chapter on small islands and its value is at once apparent.

There are a few confusing statements and discussions. On page 16, *Arbutus unedo* is correctly stated to grow "wild" in western Ireland, yet on page 17 its morphology and phenology are given as evidence that it is "not native". Could the same be said of *Hedera helix*? The discussion of interaction of factors and limiting factors on page 30 suggests a fundamental misunderstanding in the authors' minds. The text is almost free of trivial errors but the caption on Fig. 17 implies a knowledge of German which is scarcely appropriate for those who aspire to be the heirs of Alexander von Humboldt.

C. D. PIGOTT

Alpha rhythm from eyes

The Origin of the Alpha Rhythm. By O. Lippold. Pp. ix+267. (Churchill Livingstone: London and Edinburgh, 1973.) £6.

THE alpha rhythm of the electroencephalogram consists of electrical oscillations at about 10 Hz which can be recorded from the scalp, especially the occipital scalp, in most people during most of the time that their eyes are in darkness. Since the work of Adrian and Matthews in 1934 it has been almost universally believed that it is generated by the brain. Dr Lippold proposes the revolutionary idea that it is generated by muscular tremor in the orbits. The evidence comes chiefly from the following three observations. (1) A tremor of the eyes can be recorded which correlates fairly well with the alpha waves. (2) If the amplitude of the corneo-retinal potential is made different in the two eyes by dark-adapting one eye, the alpha waves from the occiput become larger on the side where the corneo-retinal potential is larger. (3) If one orbit is cooled and the other warmed (thereby presumably raising the

tremor frequency on the warmed side and lowering it on the cooled side), the alpha waves become of higher frequency on the warmed than on the cooled side.

If these observations are strictly and regularly true, I do not think that Dr Lippold's conclusion can be escaped. I therefore eagerly await their independent confirmation.

The implications of the conclusion for the conduction of current within the brain are remarkable. It is somewhat surprising that electrical waves generated in the orbit can appear much larger in records from the occipital than from the frontal scalp. It is much more surprising that they can be very large in direct bipolar recordings from the occipital cortex and undetectable in direct bipolar recordings from the precentral gyrus, only a few centimetres away and slightly nearer to the orbital fissures and optic foramen. Yet this, according to Jasper, is true of alpha waves.

G. S. BRINDLEY

Through the Earth

Physics of the Earth and Planets. By A. H. Cook. Pp. x + 316. (Macmillan: London and Basingstoke, September 1973.) £8.50.

THE author states that this book was written for second and third year undergraduate geophysics students and as background reading for graduate students. He defends the bias given to such topics as gravity, seismicity and certain dynamical behaviour of the planets on the grounds that present day students know more modern than classical physics. But the bias is rather narrower than this and the author seems to be only really sympathetic to those parts of earth science that make great play with displays of significant figures. How else is one to interpret the sentence (page 33) "The shape of the solid surface of the Earth is very irregular and of no general scientific interest"? Although there are chapters devoted to magnetism and crustal movement and some extremely sketchy descriptions of apparatus, my overall impression is of a book that could serve as an introduction to Sir Harold Jeffreys's *The Earth*.

There is a good introduction but later, in perhaps more important matters of detail than the consistent use of SI units, the standard of presentation becomes less meticulous than Sir Harold's. For example Laplace's equation is said to be "nothing more than a statement of the inverse square law" on page 27, whereas on page 28 that Laplacian fields are said to vary as r^{-2} . There follows a repetitious discussion of satellite motion and a treatment of geoidal shape, complicated

enough at the best of times, that is marred by sign errors. There are other indications of hasty preparation and inadequate proof reading. Why give a glossary of symbols when additional definitions (for example of m) are introduced into the text? Why give two diagrams of travel-time curves (pages 75 and 78) when one suffices and is there any need, even in a student text, to give diagrams of upper mantle velocity distributions (page 84) that conceal quite so many complexities of the observed situation?

There follow chapters dealing with oscillations and vibrations, the internal structure of the Earth (which ends with an entirely theoretical discussion of creep and the Q of materials), the radioactivity and ages of rocks and the temperature within the Earth. The latter chapter follows entirely conventional lines, based on thermal conduction theory although we are warned (page 171) that such calculations only apply to those parts of the Earth that are "rigid". Having been told in a previous chapter about the creep of rocks and that most of the mantle is at temperatures at which creep is "almost certainly important" (page 136), the student will wonder about the consistency of pursuing this particular line of enquiry. A quite erroneous description is given of phonon thermal conductivity (page 174), valence bands are described as localised electron states (page 175) and exciton energy transport is said to dominate other modes of the heat conduction process (page 176). A completely garbled account is given of the fundamentals of thermal convection theory, and the matter is not clarified by a quite incomprehensible fig. 7.11. I was very irritated by the implication that all is still confusion and ignorance in such an important problem of planetary physics, where definite progress has been made in the past ten years.

The remaining chapters deal with the permanent magnetisation of rocks, the geomagnetic field, movements of the Earth's crust and "The Earth among the Planets", the latter being largely concerned with the constraints placed on the density distributions in planets by classical astronomical observations and theoretical equations of state. Little reference is made to the new insights that have sprung from the space programme.

It is perhaps no criticism of a book intended for students that there are so few actual references to the work of living earth scientists, although a few names are dropped from time to time. Perusing the biographical notes at the end of the book, a student could be forgiven for believing that planetary science was the exclusive preserve of titled British scientists and finished with

the death of that well known geophysicist, Lord Rutherford. He will however find the questions at the end of most chapters a challenge to his understanding—even if they are a little contrived.

Since the publishers have performed their side of the production so satisfactorily, it is a pity that so much is unsatisfactory in its contents. I fully appreciate how difficult it is to be the expert in all branches of planetary science that such a title demands, but a little more care in preparation could have made this a much better book.

D. C. TOZER

Too brief a tale

A Revolution in the Earth Sciences: From Continental Drift to Plate Tectonics. By A. Hallam. Pp. vii+127. (Clarendon: Oxford; Oxford University: London, April 1973.) £4 boards; £1.75 paper.

THIS is a narrative historical account of the concept of continental drift. It is written in much too short a space to tell a story so deeply enmeshed in personality and full of drama. Dr Hallam deliberately aims to write "quite sparingly" and his book thereby takes on an encyclopaedic character. All the principal papers are described, though often in no more than abstract form, but the background is largely missing. He tells what was written, but not what was thought. Nor does he explain the extent of knowledge and ignorance at crucial times in the narrative.

Dr Hallam has painted a stark and static picture of the players. He has barely painted the scenery at all.

DAVID DAVIES

Substitutions

Aliphatic Nucleophilic Substitution. By S. R. Hartshorn. Pp. viii+172. (Cambridge University: London, November 1973.) £3.80; \$12.50.

THIS book is a useful member of the series of chemistry texts published by the Cambridge University Press and is certainly worth a place in any college or university library.

The author has given a clear account of nucleophilic substitution; the various methods of determining the type of reaction are discussed in detail and the problem of the "borderline" cases emphasised, an important feature since many undergraduates fail to appreciate this problem and believe that all substitution reactions fall into clear-cut categories. I was very pleased to see a section on the catalysed reactions, as the effects of silver and mercury salts on these reactions are often ignored.

In other places the treatment seems a little thin: for instance, eight pages of the book are devoted to secondary deuterium isotope effects and yet ambivalent nucleophiles only merit half a page and Kornblum's name does not appear in the list of references. Some space should have been used to discuss the important synthetically useful reactions and their limitations: dozens of graduates go out into the world each year believing that *t*-butyl bromide plus sodiomalonate ester constitutes a useful synthetic reaction. In the index there is no reference to diethyl malonate or β -keto esters and the list of solvents does not include dimethyl sulphoxide.

A. S. BAILEY

Techniques for structure

Co-ordination Chemistry: Experimental Methods. By K. Burger. English translation edited by Ian T. Millar and D. W. Allen. Pp. 372. (Butterworth: London, 1973.) £10.

TITLES must be brief, so may be incomplete. This book describes experimental methods for providing structural information in coordination chemistry and does not cover measurements of equilibrium constants or the kinetics of complex formation. In a masterly introduction the author sets the perspective and in his final, the eleventh chapter, he shows with examples from glyoxime complexes how the various methods can be combined.

The intervening chapters each deal with specific techniques giving the principles, an outline of the methods and the type of information obtainable. The balance has been influenced partly by the length of time for which the technique has been available; Mössbauer spectroscopy has advanced so much since the Hungarian edition was published in 1967 that the chapter on it was rewritten and is now as long as that on IR and Raman spectroscopy despite their more general applicability. Post 1967 references are rare in the rest of the book, and the new technique, electron spectroscopy for chemical analysis (ESCA), provides most of these.

There is a particularly clear explanation of shielding effects in the chapter on nuclear magnetic resonance (by L. Brajer). In the first three sections on X-ray structure analysis (by Korecz) there is confusion between atoms and lattice points while the illustration of an electron density map, also on the dust jacket, is not of a coordination compound; Burger himself writes well on the results. An interesting account of thermal analysis (by G. Liptay) shows that this must be combined with other methods if structural information

is sought. The use of optical rotation and circular dichroism in stereochemistry are well described and the account of magnetic methods presents spin-orbit coupling with unusual clarity.

The book succeeds in its aim, to enable someone starting research to choose an appropriate technique for the problem. It is also readable and sufficiently clear to help someone struggling with obscurities in a more specialised text. The references and examples cover a wider range than the monolingual scientist usually meets, as shown by the author index which, with a subject index, is another good feature. The standard of printing and illustration is good; unfortunately few research students will be able to afford their own copies.

MARY R. TRUTER

Neurones connecting

Developmental Neurobiology of Arthropods. Edited by D. Young. Pp. vii+268. (Cambridge University: London, December 1973.) £5.60.

THE editor of these nine experimental and review contributions has wisely declined to erect principles or to look too hard for coherence within the phenomena covered by the volume. Such treatment might have exerted an intellectual 'closure' effect on a field involving many young as well as more experienced workers. Instead, the impression is preserved that contributors are usually defining questions which may in future be posed, using their experimental systems. The book is thus good reading for intending researchers and teachers, and not recommended for those in search of potted literature from which to bolster grand theory.

Readers with developmental interests, and vertebrate research experience, spend much of their time fascinated by features distinctive to arthropod as opposed to vertebrate systems, particularly the relatively small number of neurones, and the high proportion of these which seem to be uniquely specified and 'homologous' anatomically as between individuals. But they are continually reminded that the anatomical picture of a lower order of determinacy within vertebrate nervous systems does not itself rule out a comparable degree of cell specificity at some functional level. Thus arthropods may lend themselves well to developmental analysis while contributing greatly in future to knowledge of general principles underlying neural organisation, where such exist. Both aspects are stressed at points in the book. The concluding review by Horridge contains discussion of uniqueness among neurones, and the editor in his own contribution makes some very thought-provoking remarks (page 186)

on the homology concept as applied to cells within successive segmental ganglia.

Levi-Montalcini and her associates provide a rich source of technical information for initiates into insect nervous system tissue culture, together with evidence for specificity capacities of growing neurites that has so far emerged from such work.

Bate and Lawrence review the theory, concerning spatial organisation in insect development generally, with which the latter is associated, and then suggest its relevance to such organisation as studied in the eye-brain connections of vertebrates. They then show how a particular piece of specificity in sensory behaviour, emerging in an insect pupal stage, is explicable if different receptor cell-bodies form central connections according to their position in a quantitative antero-posterior gradient of positional information that controls epidermal pattern formation in general. In such a cross-disciplinary publication as this they might have discussed alternative theories available for positional information control, but the important point is made that if position within a cell array is signalled quantitatively only, then competitive mechanisms are strongly implicated in normal erection of neuronal projections. Edwards and Palka in fact offer evidence of synaptic competition during development, in their chapter on sensory regeneration.

The insect eye—optic lobe system, described by Meinertzhagen, deserves detailed acquaintanceship as perhaps the largest array of neurones known in which many are completely specified as to anatomical class and connectivity. Although making demanding reading, this chapter is perhaps the most exciting for the relative newcomer to arthropod neurobiology.

Pipa and Bentley each provide surveys of post-embryonic development, from functional and anatomical viewpoints, and Hoy introduces the rather outlandish properties of regeneration and survival in certain crustacean axons.

JONATHAN COOKE

Associates of viruses

Viruses and Invertebrates. Edited by A. J. Gibbs. Pp. xvi+673. (Frontiers of Zoology vol. 31.) (North-Holland: Amsterdam and London; American Elsevier: New York: 1973). Dfl 150; \$57.70.

THE editor of this book believes that it is vital to develop a unified approach to virology, to counteract a bigoted adherence to an order of events based on the taxonomic position of the host. This philosophy deserves active support. The topic of 'viruses associated with invertebrates' was a particularly good choice as it allows attention to be focussed on

both animals and plants. The enormous scope of the subject is however, demonstrated by the fact that the editor needed the assistance of thirty-three authors who contributed thirty-one chapters. It is understandable that, with so many collaborators, not only will variable standards of treatment and presentation arise but that dangers of superficiality and irrelevance may occur. But with one or two exceptions, Dr Gibbs has kept his troops under disciplined command and produced a valuable and stimulating book.

The names of the various sections owe more to the stage and art than to science and yet they stimulate interest in the contrasts and direction of thought contained within and between the various chapters. For example, to continue with the artistic analogy, "Tryptych" leaves an impression of a miniature dominated by two massive canvasses. The quality of the paper is poor: it is so thin that photographs on the reverse side of a page become obtrusive. Also, a general index of only two pages is cursory and one has to rely on the brief contents list of each chapter. The price of the book is high and it will probably only find its way into libraries, but it is worth borrowing.

T. W. TINSLEY

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Is space time?

Space and Time in the Microworld. By D. I. Blokhintsev. Pp. xiv+330. (Reidel: Dordrecht and Boston, Mass., 1973.) Dfl. 110; \$39.50.

BOTH relativity and quantum theory profoundly alter Newtonian concepts of space and time. Each theory introduces changes that are qualitatively different and their compatibility has been the topic of many debates. In special relativity, the individual process is still analysed in terms of point particles moving along trajectories in a manifold, although the interactions are always propagated with a bounded finite velocity. Quantum theory, on the other hand, only gives meaning to the individual process in terms of the ensemble and, furthermore, there seems to be an essential connectedness between interacting systems that are eventually spatially separated, giving rise to a correlation that seems to be non-local.

In the non-relativistic domain we know how to accommodate the quantum theory; the classical analogue of the phenomena can be successfully translated into the quantum algorithm, but once we move into the high energy region, the situation becomes somewhat confused. Does the essential connectedness of quantum theory imply that space-time descriptions are futile and that Lorentz invariance should be introduced through energy-momentum considerations alone or should we ask questions about possible modifications to space-time geometry? Indeed, the aim of Professor Blokhintsev's book is to present a critical analysis of this type of question.

He begins by developing the mathematical description starting from the Minkowski formalism of space-time, passing briefly through field theory, finally arriving at the S-matrix formalism which is to form the basis for the rest of the investigation. In fact, he goes as far as to claim that the S-matrix will be retained in all future theories, although the methods of constructing the matrix elements may undergo radical changes.

His main thesis then centres around the question of causality. In particular, he considers the consequences of replacing microscopic causality by a less restrictive form of macroscopic causality. This allows some form of acausality, or non-locality, in small space-time regions, while retaining causality in the large. The S-matrices constructed using either principle of causality differ only at energies that are much greater than the reciprocal of a fundamental length. Experiments show this to be less than 10^{-14} cm and the author speculates that it could be 10^{-16} cm, the characteristic

weak-interaction length. But the arguments are slender and it could equally well be at 10^{-33} cm, the gravitational length, in which case further investigation might be discouraged. It has, however, often been suggested that the infinities in both classical and quantum field theories could be removed by changing the geometry in the small and the advantage of the principle of macroscopic causality is that it allows an exploration of various possibilities without leading to large-scale acausality. The final chapters are concerned with this question and discuss, in particular, the effects of discrete space-times, stochastic space-times and fluctuating geometries.

These final chapters left me feeling a little disappointed that more space had not been given to the question of generalised geometries. I find this a very fascinating field in which many questions are still unanswered. Indeed there is quite an extensive literature on the subject some of which is quite closely related to the topics discussed in the book but unfortunately no reference is made to this work. Apart from this small reservation, I find the book very clearly written, and even the liberal use of mathematics has not been allowed to obscure the essential questions.

B. J. HILEY

All sorts of skin

The Integument. A Textbook of Skin Biology. By R. I. C. Spearman. Pp. vi+208. (Biological Structure and Function, vol. 3.) (Cambridge University: London, June 1973.) £4.20; \$12.95.

BECAUSE of the explosive developments taking place in science in general and biology in particular and because of the trend toward more and more specialisation, books which give a broad, comprehensive view of the subjects included under these headings have become a rarity. Thus, I began to read this book with some anticipation which was further whetted by the author's commitment to provide a bird's eye view of the whole subject of skin biology. As the author states in his preface, the comparative approach to the study of the outer covering of both invertebrates and vertebrates has not been attempted before; hence any book purporting to do so should be expected to fill a long-felt need. Unfortunately, *The Integument* fails to live up to these expectations and does little more than catalogue the various aspects of skin biology under various headings.

The book is divided into two parts, the first of which treats the comparative morphology of the integument of both invertebrates and vertebrates. Here

Spearman deals with the epidermal, dermal and pigmentary aspects of the integument of various phyla of invertebrates and of five classes of vertebrates (Pisces, Amphibia, Reptilia, Aves and Mammalia). This section is nicely illustrated with numerous line drawings which should help the beginner to understand the structural details of many of the skin appendages.

In the second part, the book deals with the comparative functions of skin, such as thermal regulation, chemical and neural control mechanisms, comparative synthetic processes, transport through the skin, immunity and development. The titles are provocative, but the cursory treatment leaves much to be desired. For example, in the chapter on the comparative synthetic process, only ten pages are devoted to such varied topics as genetic code, protein syntheses, elastin, resilin, collagen, and a host of others.

Brevity may be the soul of wit, but since wit is hardly the reason for undertaking a study such as this, the kind of one-sentence treatment accorded to important topics by Spearman while serving to whet the appetite does little to allay the hunger for substantial data.

PAUL PARAKKAL

Kidney disease

Renal Histopathology: A Light Microscopy Study of Renal Disease. By Robert Meadows. Pp. xii+363. (Oxford University: London and New York, December 1973.) £14.

WITHIN the limits of the title Dr Meadows has written an interesting and very well illustrated book. It will be useful to all pathologists who examine renal biopsies and to the many physicians who seek to understand the structural changes in their patients. While agreeing with the author that most diagnoses can be achieved with the light microscope, fortified by knowledge obtained by others with ultrastructural and immunofluorescent methods I feel it is a pity that there is not more reference to these in the text and some illustrations of the immunofluorescent results which after all are obtained with the light microscope.

The consideration of glomerular changes and glomerulonephritis is comprehensive and clear although the separation of the two leads to a certain amount of recapitulation. The section on the disappearance of glomeruli from the kidney is welcome in that it considers a difficult point which most authors skate over. Dr Meadows is not alone in attributing the clear description of what he calls the ischaemic obsolescent glomerulus to McManus (1950); it was in fact recognised by Russell (1929) by the

less cumbersome term of ischaemic atrophy. She used the term toxic atrophy for a glomerular lesion in chronic glomerulonephritis which is redescribed in more detail by Dr Meadows who puts forward the thesis that some cases of chronic glomerulonephritis develop as a progressive form of focal disease. In her Medical Research Council Special Report (No. 142), Russell (1929) had an interesting name for this concept—nephritis repens—which has never caught on. Perhaps these reports repose in dusty basements or galleries of libraries in Australia just as they do in this country.

The chapters on tubular disease are not quite as good as those on glomerular because of a lack of adequate integration with experimental work. In understanding the changes in biopsy and necrosy material from acute tubular necrosis, for example, it is essential to appreciate the appearances of regenerating tubular epithelium at various stages—the details of which are most easily seen in experimental mercury poisoning. The only consideration of such regeneration is confined to ischaemic tubular necrosis and the sequence is not brought out. The part which obstruction may play in the pathogenesis of tubular damage is not discussed; in this respect it is unfortunate that the paper of Bywaters (1946) in which he brought out the resemblance between changes in acute hydronephrosis and the crush syndrome has never received proper recognition.

J. F. SMITH

Pictures of viruses

Ultrastructure of Animal Viruses and Bacteriophages. An Atlas. Edited by A. J. Dalton and F. Hagenau. (Ultrastructure in Biological Systems vol. 5.) Pp. xii+413. (Academic (Harcourt Brace Jovanovich): New York and London, September 1973.) \$37.50.

THIS book contains a splendid collection of electron micrographs illustrating the structure and development of 65 animal viruses and bacteriophages. Though the text is variable from chapter to chapter it is mostly concisely informative. The index is very comprehensive but it did not include the Sendai virus, which is now much used in cell fusion studies.

The chapters are divided according to the virus groupings laid down by the International Committee on Nomenclature of Viruses (ICNV). Each begins with a concise account of the structure of the virion and of its intracellular replication. As might be expected the text is not a comprehensive treatise on each group of viruses nor is each group uniformly treated. For instance R. W. Horne and B. Roizman each devote nine pages to their respective groups the adenoviruses and the herpesviruses,

whereas N. Higashi and A. J. Dalton fill only one page each about the togaviruses and the arenaviruses. Nevertheless all the chapters have useful lists of references.

Several authors have been particularly helpful by giving clear accounts of more obscure aspects of their chosen group. A. J. Dalton and F. Hagenau define the A, B and C particles found in neoplastic tissues and G. C. Goodman gives a detailed account of the cytopathic events in cells infected with picornavirus in the captions to the illustrations. J. L. Melnick gives an account of the efforts of the ICNV to name and classify the viruses.

It is a pity that a book which treats the animal viruses so fully should expend two chapters on bacteriophage but exclude the viruses of plants and invertebrates. But the book completely achieves its editors' aims, provides a valuable work of reference for the animal viruses and gives a concise view of the bacteriophages.

D. KAY

Cosmic rays reach earth

Cosmic Rays at Ground Level. Edited by A. W. Wolfendale. Pp. vii+232. (Institute of Physics: London and Bristol, 1973.) £12.00.

As a tribute to Professor G. D. Rochester on the occasion of his retirement, nine authors associated with the cosmic ray group at the University of Durham have chosen to write ten short reviews, in which they have summarised the present situation in experimental work on the fluxes of the various components of the secondary cosmic radiation detected at "ground level", in chapters on energetic muons (M. G. Thompson), protons and pions (Brooke), neutrons (Ashton), μ -neutrinos (Osborne), e -neutrinos (E. C. M. Young), extensive air showers below 10^{17} eV (Wdowczyk) and above 10^{17} eV (Turver), and on searches for quarks and other hypothetical particles (Ashton). The work is not about the primary cosmic radiation, although for completeness a preliminary chapter (Wolfendale) sketches in some of its aspects. There is added a comprehensive account of the technique of observing trajectories of fast charged particles by neon flash tubes (Breare), a method used by most of the authors.

In several chapters the aim is principally to collect data on the particle fluxes near sea level, and the "ground" in the terms of reference strictly excludes mention of experiments at mountain altitudes: hence a theoretical framework for interpreting the observations is not the aspect of interest. In the circumstances it is natural that the authors, apart from collecting published data, present progress reports which tend to lean heavily

for illustration on the experiments in which they and their colleagues took part, in the research groups founded by Rochester and Wolfendale; although extensive references are given to other experiments.

In the most substantial review, on observations of momentum spectra, angular distributions and charge ratios of energetic muons, readers with a peripheral interest in the subject may be surprised by the extent to which for many years results have depended on normalisation to an old experiment by Greisen: only recently has the normalisation been revised (by Allkofer and others), and it is useful to have this documented. (It is a pity though that the "ground level" rule bars any reference to the work of the Utah group.) Although some problems currently raised by the interpretation of the charge ratio are briefly introduced, the significance of the vertical momentum spectrum is not discussed. The reviewers of hadrons have very little to work with at energies above a few GeV, mountain experiments having been more numerous, and the distinction between pions and protons (at sea level) evidently remains a problem. Here, though, a discussion of the method of deducing the energies of hadrons in the TeV range from the cascades they produce would have been welcome, as different experiments are almost certainly not using the same scale. (In the short chapter on neutrons, the method used to measure their spectrum is not mentioned at all.) And for all very high energy spectra, one would have liked to know how much accompanying particles might affect the measurements.

To observe neutrinos one has to go to deep mines, but with the excuse that the fluxes there and at sea level must be the same, Osborne gives a clear account of the three main experiments on μ -neutrinos, and their significance. Young and Ashton have no positive observations to report on their exotic particles, but they indicate what intensities might reasonably be sought. In the articles on extensive air showers the emphasis shifts to illustrating progress being made by the authors and their colleagues in constructing models of shower development, which is a necessary preliminary to firm deductions about the energies and nature of the primary particles; although Turver also presents recent estimates of the energy spectrum, and isotropy, and recent work on the distribution of several particle components of air showers, largely from the Haverah Park experiments.

The fluxes of electrons, gamma rays and slow neutrons are not reviewed. It is a pity that the price is so high for a welcome but relatively short book.

A. M. HILLAS

matters arising

T-mycoplasmas and infertility

SIR,—IN the first report about the association of T-mycoplasmas with human infertility the authors considered that the high incidence of isolation (89%) from the cervix of infertile women compared with the low incidence of isolation (23%) from pregnant women suggested that the T-mycoplasmas were playing some rôle in the infertility. This was based on the isolation discrepancy and was principally a result of the low incidence in pregnant women. Others have, however, been more successful in isolating T-mycoplasmas from pregnant women. Mårdh and Weström² isolated T-mycoplasmas more frequently from pregnant (68%), than from non-pregnant women (46%), and thought that the former rate might have been due to changes in the genital tract at pregnancy conducive to the growth of T-mycoplasmas. Gnarpe and Friberg tried to substantiate the possible association of T-mycoplasmas with infertility by treating infertile couples with tetracycline and 29% of the women they treated subsequently became pregnant. In the absence of untreated controls, however, this finding is meaningless. Mr G. T. Smedley and I, in an unpublished study, found that of 32 infertile women, 22 (69%) carried T-mycoplasmas. Within a year, nine of these women became pregnant without treatment and 5 (56%) of them carried T-mycoplasmas. Gnarpe and Friberg's second report³ concerns the attachment of T-mycoplasmas to human spermatozoa. I find it difficult to regard the 'blobs' on the spermatozoa that they saw by electron microscopy as evidence for attached T-mycoplasmas, as these structures could also be *Mycoplasma hominis*, chlamydiae or some other agent. I do agree, however, that adherence of human T-mycoplasmas occurs in the same way as those of bovine origin adhere to bovine spermatozoa⁴. It is interesting that practically all bovine semen samples that we examined contained T-mycoplasmas, yet they all had a high fertilising capacity⁵. It seems likely that abnormal appearance, low motility and viability of spermatozoa in man are not due to the presence of T-mycoplasmas in semen, because the organisms are isolated from such samples no more frequently than from samples which contain apparently normal spermatozoa. Spermatozoa of low fertilising potential might also be

adversely affected by mycoplasmas present in the vagina and cervix. This comes back to the original point about the incidence of T-mycoplasma isolation from these sites. Whether the incidence of isolation is greater in infertile women is a moot point, but it would also be interesting to know whether they harbour more organisms in the genital tract than do fertile women. I feel that the evidence put forward to incriminate T-mycoplasmas as a cause of infertility is far from convincing and that they are being placed on a pedestal that they do not, so far, deserve.

Yours faithfully,

D. TAYLOR-ROBINSON

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¹ Gnarpe, H., and Friberg, J., *Nature*, **242**, 120 (1973).

² Acta Path. Microbiol. Scand., **78**, 367 (1970).

³ Gnarpe, H., and Friberg, J., *Nature*, **245**, 97 (1973).

⁴ Taylor-Robinson, D., and Manchec, R. J., *Mycoplasma Diseases of Man*, Veb Gustav Fischer Verlag, Jena, p. 113 (1969).

⁵ Taylor-Robinson, D., Thomas, M., and Dawson, P. L. J. Med. Microbiol., **2**, 527 (1969).

DRS GNARPE AND FRIBERG REPLY: we have read with interest the above letter and although Dr Taylor-Robinson's comments are valuable we have by no means expressed the opinion that T-mycoplasmas might be one of the major causes of infertility.

There are, however, a few points we wish to make clear. In our first investigation¹ we found a high incidence of T-mycoplasmas in infertile men and women compared with a lower incidence in men and women with proven fertility. All infertile patients had an unexplained infertility of 5 yr or more. None of the spermatozoa specimens investigated had abnormal appearance under light microscopy, low motility or reduced viability. We still consider that the incidence of pregnancy after tetracycline treatment is higher than can be expected to appear by chance among patients with this long-standing infertility².

We agree with Dr Taylor-Robinson that the 'blobs' on human spermatozoa discovered in scanning electron microscopy might be other agents such as *M. hominis* or Chlamydiae. In all specimens where cultures were made of

thoroughly washed spermatozoa³, however, we found T-mycoplasma colonies and not other agents growing. Whether Chlamydiae were present or not was, of course, impossible to establish with the technique used. We also found that some T-mycoplasma isolates produced substances cross-reacting with neuraminidase antibodies and suggested that these substances might render conception more difficult³.

These findings make it possible that some T-mycoplasmas might be of importance for some cases of unexplained infertility.

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¹ Gnarpe, H., and Friberg, J., *Nature*, **242**, 120 (1973).

² Friberg, J., and Gnarpe, H., *Am. J. Obstet Gynec.*, **116**, 23 (1973).

³ Gnarpe, H., and Friberg, J., *Nature*, **245**, 97 (1973).

African swells, magmatism and plate tectonics

SIR,—Some of the 'flaws'¹ in my lateral compression model² seem to be matters of faith, for example, that the model is "at odds with modern global tectonics", whilst many (including the "petrological observations") are arguable matters of interpretation. One "flaw", apparently supported by data, concerns the faster rate of uplift of post-glacial regions compared with the doming of East Africa. It is argued that since there is no evidence of deep partial melting in the post-glacial regions, the probability of such melting by warping of the African craton is insignificant. But the two mechanisms are quite different. The post-glacial regions are rising in response to a uniform isostatic pressure at the base of the plate. Below the post-glacial uplift there is no localised decompression, as might be the case below an 'upwarp'. As was pointed out in 1964 (ref. 2), in the case of the African swells "lighter material would have to be added at depth to compensate each succeeding uplift". So far as I am aware, this deduction has not been challenged, nor an alternative offered.

In any case, since 1964 my original hypothesis² has been considerably modified and refined³, and Gill's critique is

therefore something of an anachronism. In particular, the interested reader will find³ the evidence and arguments against the idea (said by Gill to be "widely regarded") that the "topography of Africa is . . . the product of heat flow anomalies in the upper mantle". An alternative is proposed which is more in keeping with the facts.

Yours faithfully,

D. K. BAILEY

Department of Geology,
University of Reading

¹ Gill, R. C. O., *Nature*, **247**, 25 (1974).

² Bailey, D. K., *J. geophys. Res.*, **69**, 1102 (1964).

³ Bailey, D. K., *J. Earth Sci.*, **8**, 225 (1972).

Announcements

Appointments

The Council of the University of Manchester has appointed **E. R. Trueman** to be Beyer Professor of Zoology. **D. M. Guthrie** has been appointed to be a Professor of Zoology in succession to Professor Trueman.

The British Library Board has appointed **D. Richnell** as Director General of The British Library, Reference Division.

J. Latham has been appointed to a Chair in Physics and **D. S. L. Cardwell** has been appointed to a Chair in the History of Science and Technology at the University of Manchester Institute of Science and Technology.

Awards

The **Edinburgh Geological Society** has awarded the **Clough Medal** to **Dr Dorothy H. Rayner**, for her contributions to geology.

The **Zoological Society of London** has awarded the **Scientific Medal** to **Dr J. N. Brady** of the Imperial College of Science and Technology, London, and **Professor H. C. Macgregor**, of the University of Leicester; the **Stamford Raffles Award** to **Mr G. H. Lockett**; and the **Thomas Henry Huxley Award** to **Dr Patricia Farquharson** of Westfield College, London.

The **Biochemical Society** has awarded the **Colworth Medal** for 1973 to **J. C. Metcalfe** of the Department of Pharmacology, Cambridge; the **CIBA Medal and Prize** to **P. D. Mitchell** of the Glynn Research Laboratories, Bodmin; and the **Keilin Memorial Medal** to **E. C. Slater** of the BCP Jansen Institute, University of Amsterdam.

International Meetings

March 28, Solids Handling and Metering associated with an NPK Prilling Plant (J. C. Pinder, The Fertiliser Society, Alembic House, 93 Albert Embankment, London SE1 7TU)

March 31–April 12, Cours de formation sur la recherche anticancéreuse (Dr J. F. Delafresnaye, Union Internationale Contre le Cancer, Rue du Conseil-Général 3,1205 Geneva, Switzerland)

April 1–2, Gene Isolation and HnRNA (Dr J. P. Goddard, Organising Secretary, Nucleotide Group Meeting, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ)

April 1–2, Synchrotron Radiation and its applications to the Analysis of Problems in Scientific Investigation (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX)

April 1–3, The Design of Scientific and Technical and Scientific Exhibitions (The Centre for Extension Studies (Z), University of Technology, Loughborough, Leicester LE11 3TU)

April 1–3, Electrets and High Field Polarization (Dr C. W. Smith, Meetings Secretary, Dielectrics Discussion Group, (Dielectrics Society), University of Salford, Department of Electrical Engineering, Salford M5 4WT, UK)

April 1–4, 5th International Geochemical Exploration Symposium (John Barakso, c/o Mineral Environment Laboratories Ltd., 705 West 15th Street, North Vancouver, British Columbia, Canada)

April 1–5, Conference on Animal Feeds of Tropical and Sub-Tropical Origin (Public Relations C, Tropical Products Institute, 56-62, Gray's Inn Road, London WC1X 8LU)

April 1–5, Basic Electron Microscopy Course for Biologists and Materials Scientists (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA)

April 1–5, Radio: Roads, Tunnels and Mines (Institut National des Industries Extractives, rue du Chera, B-4000, Liege, Belgium)

April 1–5, Post-Graduate School on Pharmacokinetics (Mr R. E. Marshall, School Secretary, Department of Pharmaceutical Sciences, The Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London WC1A 2NN)

April 1–5, International Symposium on Advances in Polymer Friction and Wear (Dr Lieng-Huang Lee, Symposium Chairman, Wilson Center of Technology, Xerox Corporation, Webster, New York 14580)

April 1–5, Annual Chemical Congress of the Chemical Society and the Royal Institute of Chemistry (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK)

April 1–6, Symposium on Afar (Ethiopia) and Related Rift-Structures (Professor A. Pilger, Geologisches Institut der Technischen Universität Clausthal, 3392 Clausthal-Zellerfeld, West Germany)

April 2–3, Sira Conference on Quality Control (Mrs Ruth Keiller, Sira Institute Ltd., South Hill, Chislehurst, Kent BR7 5EH)

April 2–4, 2nd International Symposium on Jet Cutting Technology (The Organising Secretary, 2nd ISJCT, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK)

April 2–4, The Mechanical Properties of Materials at High Rates of Strain (Dr J. Harding, Department of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PJ) (Changed from 19–21 March)

April 2–4, Multi-Phase Flow Systems (Department NTS, The Institution of Chemical Engineers, 16, Belgrave Square, London SW1X 8PT, UK)

April 3–4, Colloquium on Molluscan Phylogeny (Dr N. J. Morris and Dr J. D. Taylor, British Museum (Natural History) Cromwell Road, London SW7 5BD)

April 3–4, Metal Semiconductor Contacts 9B. (L. H. Wilson, The Plessey Co Ltd., Allen Clark Research Centre, Caswell, Towcester, Northamptonshire)

April 4–5, 547th Meeting of the Biochemical Society (Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP)

April 4–6, 6th Conference of the Institute of Information Scientists (The Conference Secretary, Mrs S. E. Stevenson, Solartron Electronic Group Ltd., Victoria Road, Farnborough, Hampshire)

April 5, Spring Meeting of the Clinical Genetics Society (Dr Derek A. Smith, Genetics Department, University of Birmingham, P.O. Box 360, Birmingham 15)

April 5–9, Franco-British Centenary Conference (The Meetings Officer, Institute of Physics, 47, Belgrave Square, London SW1X 8QX, UK)

April 8–10, National Engineering Laboratory and Society for Underwater Technology International Symposium on Exploitation of Vibration (Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 0QU)